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(54) Title: ANTIBACTERIAL TARGETS IN ALLOIOCOCCUS OTITIDIS

(57) Abstract: The present invention relates to the identification of polynucleotide sequences encoding polypeptides of *Alloiococcus otitidis* that are essential for the growth and survival of the bacteria. In particular, the invention relates to polypeptides encoded by the *Alloiococcus otitidis* open reading frames (ORFs), and to their use in pharmaceutical compositions, therapeutics, diagnostics and the like. The present invention also relates to methods for identifying pharmaceutical compounds that inhibit the activity of the polypeptides that are essential for the growth of *Alloiococcus otitidis*, to pharmaceutical compositions containing these compounds and to their use in treatment and amelioration of diseases caused by *Alloiococcus otitidis*.

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ANTIBACTERIAL TARGETS IN ALLOIOCOCCUS OTITIDIS

FIELD OF THE INVENTION

5 The present invention relates to the genomic sequence of *Alloiococcus otitidis* and polynucleotide sequences encoding polypeptides of the Gram-positive bacterium, *Alloiococcus otitidis*. The invention also relates to polynucleotides and polynucleotides encoding polypeptides, preferably antigenic polypeptides, encoded by the *Alloiococcus otitidis* open reading frames and the uses thereof.

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BACKGROUND OF THE INVENTION

Since the discovery of penicillin, the use of antibiotics to treat the ravages of bacterial infections has saved millions of lives. With the advent of these "miracle drugs," for a time it was popularly believed that humanity might, once and for all, be saved from the scourge of bacterial infections. In fact, during the 1980s and early 15 1990s, many large pharmaceutical companies cut back or eliminated antibiotics research and development. They believed that infectious disease caused by bacteria finally had been conquered and that markets for new drugs were limited. Unfortunately, this belief was overly optimistic. The tide is beginning to turn in favor of the bacteria, as reports of drug resistant bacteria become more frequent. The United States Centers for Disease Control and Prevention announced that one of the most powerful known antibiotics, vancomycin, was unable to treat an infection of the common bacterial pathogen, *Staphylococcus aureus*. This organism, commonly found in our environment, is responsible for many nosocomial infections. The import 20 of this announcement becomes clear when one considers that vancomycin was used for years to treat infections caused by *Staphylococcus* species as well as other stubborn strains of bacteria. In short, bacteria are becoming resistant to our most powerful antibiotics. If this trend continues, it is conceivable that we will return to a time when what are presently considered minor bacterial infections are fatal 25 diseases.

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Over-prescription and improper prescription habits by some physicians have caused an indiscriminate increase in the availability of antibiotics to the public. The patients are also partly responsible, since they will often improperly use the drug,

thereby generating yet another population of bacteria that is resistant, in whole or in part, to traditional antibiotics.

The bacterial pathogens that have haunted humanity remain, in spite of the development of modern scientific practices to deal with the diseases that they cause. Drug resistant bacteria are now an increasing threat to the health of humanity. A new generation of antibiotics is needed to once again deal with the pending health threats that bacteria present.

As more and more bacterial strains become resistant to the panel of available antibiotics, new antibiotics are required to treat infections. In the past, practitioners of pharmacology relied upon traditional methods of drug discovery to generate novel, safe and efficacious compounds for the treatment of disease. Traditional drug discovery methods involve blindly testing potential drug candidate- molecules, often selected at random, in the hope that one might prove to be an effective treatment for some disease. The process is painstaking and laborious, with no guarantee of success.

Newly emerging practices in drug discovery utilize a number of biochemical techniques to provide for directed approaches to creating new drugs, rather than discovering them at random. For example, gene sequences and proteins encoded thereby that are required for the proliferation of a cell or microorganism make excellent targets since exposure of bacteria to compounds active against these targets would result in the inactivation of the cell or microorganism. Once a target is identified, biochemical analysis of that target can be used to discover or to design molecules that interact with and alter the functions of the target. Use of physical and computational techniques to analyze structural and biochemical properties of targets in order to derive compounds that interact with such targets is called rational drug design and offers great potential. Thus, emerging drug discovery practices use molecular modeling techniques, combinatorial chemistry approaches, and other means to produce and screen and/or design large numbers of candidate compounds.

Nevertheless, while this approach to drug discovery is clearly the way of the future, problems remain. For example, the initial step of identifying molecular targets for investigation can be an extremely time consuming task. It may also be difficult to design molecules that interact with the target by using computer modeling techniques. Furthermore, in cases where the function of the target is not known or is

poorly understood, it may be difficult to design assays to detect molecules that interact with and alter the functions of the target. To improve the rate of novel drug discovery and development, methods of identifying important molecular targets in pathogenic cells or microorganisms and methods for identifying molecules that interact with and alter the functions of such molecular targets are urgently required.

The present invention is directed to identifying important molecular targets in a recently identified bacteria, *Alloiococcus otitidis*, which has been implicated in otitis media with effusion (OME). Otitis media, an inflammatory disease of the middle ear, is the most frequent cause of visits to pediatricians' offices in the United States (Schappert, 1991). Approximately 80% of all children experience at least one episode of otitis media by the age of three (Klein, 1994). There are three main types of otitis media: Acute otitis media (AOM), otorrhea, and otitis media with effusion (OME). *Alloiococcus otitidis* has only been associated with otitis media with effusion (OME), but this may be due to the difficulty of its detection by standard bacterial culturing methods. Its detection in the effusions is likely due to the fact that the effusions are normally sterile and few or no competing bacterial species are isolated from them. Without the interference of faster growing nasopharyngeal species, the culture plates can be incubated for the longer duration needed to detect *Alloiococcus otitidis* colonies.

Three other bacterial species are commonly isolated from middle ear effusions. These are nontypeable *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. One or more of these species have been found in one study to be associated with about 77% of all cases of OME using a PCR detection method (Post, 2000). This study did not include assaying for *Alloiococcus otitidis*, so a portion of the unaccounted cases may be due to this organism.

The bacterium *Alloiococcus otitidis* was first isolated from the middle ear fluids of 10 children in the Buffalo, NY area with persistent OME and characterized as a large catalase negative, Gram-positive cocci that tend to occur in clumps, often in tetrads. It is slow growing and requires 2 to 5 days at 37°C before colonies can be seen on sheep blood agar plates. The bacterium was named *Alloiococcus otitis* by Aguirre and Collins (1992), who showed that it was different from other known Gram-positive species based on its 16S rRNA sequence. The bacterium's name has been

changed from *Alloiococcus otitis* to *Alloiococcus otitidis*. (Hendolin, et al., (1999), and Hendolin et al., (2000)).

Several studies of the epidemiology *Alloiococcus otitidis* indicate it is associated with otitis media with effusion. These are summarized in Table 1. These studies have been done using both culture and PCR techniques. The number of cases detected by culture, as might be expected from the fastidious growth requirements of the bacterium, was less than the number detected by PCR. Assuming that the bacterium is detected more accurately by the PCR method, the bacterium is detected in between 10 and 50% of patients with OME. This frequency suggests that this organism represents a significant public health problem. Consequently, there is a need for identifying gene targets in *Alloiococcus otitidis* for the development of anti-infectives. There is also a need for compositions for diagnosing *Alloiococcus otitidis* infection.

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TABLE 1: SUMMARY OF STUDIES INDICATING AN ASSOCIATION OF ALLOIOCOCCUS OTITIDIS WITH OTITIS MEDIA WITH EFFUSION (OME).

% detected	N ^a	Method	Reference
8	200	Culture	Faden & Dryja, J. Clin. Microbiol. 27:2488 (1989)
3	100	Culture	Sih et al., ICAAC (1992)
20	25	PCR	Hendolin et al., J. Clin. Microbiol. 35:2854 (1997)
50	12	PCR	Beswick, et al., Lancet 345:386 (1999)
42	67	PCR	Hendolin, et al., Pediatr. Infect. Dis. J. 18:860 (1999)
10	49	PCR	Hendolin et al., J. Clin. Microbiol. 38:125 (2000)

^a Number of persons in study.

SUMMARY OF INVENTION

The present invention broadly relates to *Alloiococcus otitidis* genomic sequence. Particularly, the invention relates to newly identified polynucleotide open
5 reading frames (ORFs) comprised within the genomic nucleotide sequence of *Alloiococcus otitidis*, and to polypeptides encoded by the ORFs. More particularly, the ORFs encode polypeptides that are essential for the growth and survivability of *Alloiococcus otitidis*.

Thus, in certain aspects, the invention relates to *Alloiococcus otitidis* ORFs
10 that encode *Alloiococcus otitidis* polypeptides that function as enzymes in various biosynthetic pathways in the bacterium. In one embodiment, the invention relates to a purified or isolated *Alloiococcus otitidis* nucleic acid sequence comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, wherein expression of said nucleic acid is essential for
15 the proliferation of a cell. In a preferred embodiment the ORF selected from one of the odd numbered sequence listings set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105 encodes an essential gene. The essential gene and the polypeptide encoded by them include ACPS (holo-(acyl carrier protein) synthase), murF (UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diamino pimelate-D-alanyl-D-alanyl ligase)
20 murA-2 (UDP-N-acetylglucosamine 1-carboxyvinyltransferase), RpoE (DNA-directed RNA polymerase, delta subunit), rpoA (DNA-directed RNA polymerase alpha subunit), rpoC (RNA polymerase beta' subunit), rpoB (DNA-dependent RNA polymerase subunit beta), dnaB/C (DNA polymerase III delta prime subunit), gyrA (DNA gyrase A subunit), gyrB (DNA gyrase B subunit), dnaN (DNA polymerase III beta chain, folC-2 (folyl-polyglutamate synthetase), murE (UDP-N-acetylmuramoyl-L-alanyl-D-glutamyl-L-lysine Ligase), srtA (sortase), folC-1 (folyl-polyglutamate synthetase), folB (dihydroneopterin aldolase), folK (7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase), mvaS (hydroxymethylglutaryl-CoA synthase), mvaA (3-hydroxy-3-methylglutaryl-coenzyme a reductase), murB (UDP-N-acetylglucosaminyl-3-enolpyruvate reductase), mvaK2 (phosphomevalonate kinase),
30 mvaD (mevalonate diphosphate decarboxylase), mvaK1 (mevalonate kinase), coaA (pantothenate kinase), nadE (NAD⁺ synthase), murl, Glutamate racemase), folP (Dihydropteroate synthase), folA (dihydrofolate reductase), grlB (topoisomerase IV B

subunit), *grlA* (topoisomerase IV A subunit), *rpoD* (transcription initiation factor sigma), *dnaG* (DNA primase), *era* (GTP-binding protein), *norA* (drug-export protein), *polC* (DNA polymerase III, alpha subunit), *obg* (GTP-binding protein), *yphC* (similar to *Escherichia coli* GTP-binding protein Era), *dnaE* (DNA polymerase III, alpha subunit), *coaBC* (phosphopantothenoylcysteine synthetase/decarboxylase), *holA* (DNA polymerase III delta subunit), *coaD* (phosphopantetheine adenylyltransferase) *ftsZ* (Cell division protein *ftsZ*), *ftsA* (Cell division protein *ftsA*), *murG* (phospho-N-acetylmuramoyl-pentapeptide-transferase), *murD* (UDP-N-acetylmuramoylalanine D-glutamate ligase), *nadD* (nicotinic acid mononucleotide adenylyltransferase), *coaE* (dephospho-CoA kinase), *murC* (UDP-N-acetyl muramate-alanine ligase), *fmhB* FemX (factor essential for methicillin resistance), *pcrA* (ATP-dependent DNA helicase), *murA-1* (UDP-N-acetylglucosamine 1-carboxyvinyltransferase), *holB* (DNA polymerase III delta' subunit) and *dnaX* (DNA polymerase III -gamma and tau subunits).

15 In another embodiment, the invention relates to purified or isolated nucleic acid of *Alloiococcus otitidis* comprising a fragment of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, wherein said fragment is selected from the group consisting of fragments comprising at least 10, at least 20, at least 25, at least 30, at least 50 and more than 50 consecutive nucleotides of one of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

20 In yet another embodiment, the invention relates to a purified or isolated antisense nucleic acid comprising a nucleotide sequence complementary to at least a portion of an intragenic sequence, intergenic sequence, sequences spanning at least a portion of two or more genes, 5' noncoding region, or 3' noneoding region within an operon comprising a proliferation-required gene of *Alloiococcus otitidis* whose activity or expression is inhibited by an antisense nucleic acid and selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

25 In a nother embodiment, the invention relates to a purified or isolated nucleic acid comprising a nucleotide sequence having at least 70% identity to a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, fragments comprising at least 25 consecutive nucleotides selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, the nucleotide sequences complementary to one of odd numbered

sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, and the sequences complementary to fragments comprising at least 25 consecutive nucleotides of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

5 In another embodiment, the invention relates to a vector comprising a promoter operably linked to a nucleic acid encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of any one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

10 In another embodiment, the invention relates to purified or isolated polypeptide of *Alloiococcus otitidis* comprising a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or a fragment selected from the group consisting of fragments comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of one of the said polypeptides.

15 In yet another embodiment, the invention relates to purified or isolated *Alloiococcus otitidis* polypeptide comprising a amino acid sequence having at least 25% amino acid identity to a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or at least 25% amino acid identity to a fragment comprising at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

20 In one embodiment, the invention relates to a purified or isolated *Alloiococcus otitidis* polypeptide comprising selected from one of the even numbered sequences set forth in Seq. ID Nos: 2 to Seq. ID Nos: 106, wherein the polypeptide is essential for the proliferation of a cell..

25 In yet another embodiment, the invention relates to a method of producing an *Alloiococcus otitidis* polypeptide comprising introducing into a cell a vector comprising a promoter operably linked to a nucleic acid comprising a nucleotide sequence encoding a polypeptide whose expression is essential for the proliferation and viability of *Alloiococcus otitidis*, and which is inhibited by an antisense nucleic

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acid, and which is selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

In yet another embodiment, the invention relates to a method of inhibiting the proliferation of *Alloiococcus otitidis* in an individual comprising inhibiting the activity or
5 reducing the amount of a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105 or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product.

In a preferred embodiment, the invention relates to method for identifying a
10 compound which influences the activity of an *Alloiococcus otitidis* gene product , which is required for proliferation, said gene product comprising a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, said method comprising: (a) contacting said gene product with a
15 candidate compound; and (b) determining whether said compound influences the activity of said gene product.

In a preferred embodiment, the invention relates to method for identifying a compound or an antisense nucleic acid having the ability to reduce activity or level of a *Alloiococcus otitidis* gene product, which is required for proliferation, said gene
20 product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, said method comprising the steps of: (a) contacting a target gene or RNA encoding said gene product with a candidate compound or antisense nucleic acid; and (b) measuring the
25 activity of said target.

In yet another preferred embodiment, the invention relates to method for inhibiting cellular proliferation of *Alloiococcus otitidis* comprising introducing an effective amount of a compound with activity against a gene whose activity or expression is essential for cellular proliferation, and which is inhibited by an
30 antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or a compound with activity against the product of said gene into a population of *Alloiococcus otitidis* cells expressing said gene.

In a preferred embodiment, the invention relates to a composition comprising an effective concentration of an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or a proliferation-inhibiting portion thereof in a pharmaceutically acceptable carrier.

In a preferred embodiment, the invention relates to method for identifying a compound having the ability to inhibit proliferation of *Alloiococcus otitidis* cell comprising: (a) identifying a homologue of a gene or gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, in a test cell, wherein said test cell is not *Alloiococcus otitidis*; (b) identifying an inhibitory nucleic acid sequence which inhibits the activity of said homologue in said test cell; (c) contacting said test cell with a sublethal level of said inhibitory nucleic acid, thus sensitizing said cell; (d) contacting the sensitized cell of step (c) with a compound; and (e) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said inhibitory nucleic acid.

In a preferred embodiment, the invention relates to a method for identifying a compound having activity against a biological pathway required for proliferation comprising: (a) sensitizing a cell by providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding a gene product required for proliferation, wherein the activity or expression of said gene product is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, in said cell to reduce the activity or amount of said gene product; (b) contacting the sensitized cell with a compound; and (c) determining the degree to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

In a preferred embodiment, the invention relates to a method for identifying a compound having the ability to inhibit one of the *Alloiococcus otitidis* polypeptides encoded by a polynucleotide selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, and which is essential for cellular proliferation comprising: (a) contacting a cell which expresses the polypeptide with the compound;

and (b) determining whether said compound reduces proliferation of said contacted cell by acting on said gene product.

In a preferred embodiment, the invention relates to a method for identifying a compound having the ability to inhibit one of the purified and isolated *Alloiococcus*
5 *otitidis* polypeptides selected from one of the even numbered sequences set forth in Seq. ID No.: 2 to Seq. ID No.: 106, and which is essential for cellular proliferation comprising: (a) contacting the purified and isolated polypeptide with the compound *in vitro* in the presence or absence of a substrate, which is essential for the activity of the polypeptide; and (b) determining the effect of the compound on the polypeptide
10 by measuring the effect of the polypeptide on the substrate.

In a preferred embodiment, the invention relates to a compound which interacts with an *Alloiococcus otitidis* polypeptide selected from one of the even numbered sequences set forth in Seq. ID No.: 2 to Seq. ID No.: 106 and inhibits its activity.

15 In a preferred embodiment, the invention relates to a method for manufacturing an antimicrobial compound comprising the steps of screening one or more candidate compounds to identify a compound that reduces the activity or level of an *Alloiococcus otitidis* polypeptide selected from one of the even numbered sequences set forth in Seq. ID No.: 2 to Seq. ID No.: 106, said polypeptide
20 comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105; and manufacturing the compound so identified.

In a preferred embodiment, the invention relates to a compound which inhibits
25 proliferation of *Alloiococcus otitidis* by interacting with a gene encoding a polypeptide that is required for proliferation or with a polypeptide required for proliferation, wherein said polypeptide is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105, polypeptide encoded by a
30 nucleic acid having at least 70% nucleotide sequence identity to a nucleic acid encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105, a polypeptide having at

least 25% amino acid identity to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105, a polypeptide encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105.

DETAILED DESCRIPTION OF THE INVENTION

15

A. Definitions:

By "biological pathway" is meant any discrete cell function or process that is carried out by a gene product or a subset of gene products. Biological pathways include anabolic, catabolic, enzymatic, biochemical and metabolic pathways as well as pathways involved in the production of cellular structures such as cell walls. Biological pathways that are usually required for proliferation of cells or microorganisms include, but are not limited to, cell division, DNA synthesis and replication, RNA synthesis (transcription), protein synthesis (translation), protein processing, protein transport, fatty acid biosynthesis, electron transport chains, cell wall synthesis, cell membrane production, synthesis and maintenance, and the like.

By "inhibit activity of a gene or gene product" is meant having the ability to interfere with the function of a gene or gene product in such a way as to decrease expression of the gene, in such a way as to reduce the level or activity of a product of the gene or in such a way as to inhibit the interaction of the gene or gene product with other biological molecules required for its activity.

Agents which inhibit the activity of a gene include agents that inhibit transcription of the gene, agents that inhibit processing of the transcript of the gene, agents that reduce the stability of the transcript of the gene, and agents that inhibit translation of the mRNA transcribed from the gene. In microorganisms, agents which

inhibit the activity of a gene can act to decrease expression of the operon in which the gene resides or alter the folding or processing of operon RNA so as to reduce the level or activity of the gene product. The gene product can be a non- translated RNA such as ribosomal RNA, a translated RNA (mRNA) or the protein product resulting from translation of the gene mRNA. Of particular utility to the present invention are antisense RNAs that have activities against the operons or genes to which they specifically hybridize.

By "activity against a gene product" is meant having the ability to inhibit the function or to reduce the level or activity of the gene product in a cell. This includes, but is not limited to, inhibiting the enzymatic activity of the gene product or the ability of the gene product to interact with other biological molecules required for its activity, including inhibiting the gene product's assembly into a multimeric structure.

By "activity against a protein" is meant having the ability to inhibit the function or to reduce the level or activity of the protein in a cell. This includes, but is not limited to, inhibiting the enzymatic activity of the protein or the ability of the protein to interact with other biological molecules required for its activity, including inhibiting the protein's assembly into a multimeric structure.

By "activity against a nucleic acid" is meant having the ability to inhibit the function or to reduce the level or activity of the nucleic acid in a cell. This includes, but is not limited to, inhibiting the ability of the nucleic acid interact with other biological molecules required for its activity, including inhibiting the nucleic acid's assembly into a multimeric structure.

By "activity against a gene" is meant having the ability to inhibit the function or expression of the gene in a cell. This includes, but is not limited to, inhibiting the ability of the gene to interact with other biological molecules required for its activity.

By "activity against an operon" is meant having the ability to inhibit the function or reduce the level of one or more products of the operon in a cell. This includes, but is not limited to, inhibiting the enzymatic activity of one or more products of the operon or the ability of one or more products of the operon to interact with other biological molecules required for its activity.

By "antibiotic" is meant an agent which inhibits the proliferation of a cell or microorganism.

By "homologous coding nucleic acid" is meant a nucleic acid homologous to a nucleic acid encoding a gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105 or a portion thereof. In some embodiments, the homologous coding nucleic acid may have at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and fragments comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides thereof. In other embodiments the homologous coding nucleic acids may have at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequences complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and fragments comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides thereof. Identity may be measured using BLASTN version 2.0 with the default parameters or tBLASTX with the default parameters. (Altschul, S.F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, Nucleic Acid Res. 25: 3389-3402 (1997)) Alternatively a "homologous coding nucleic acid" could be identified by membership of the gene of interest to a functional orthologue cluster. All other members of that orthologue cluster would be considered homologues. Such a library of functional orthologue clusters can be found at <http://www.ncbi.nlm.nih.gov/COG>. A gene can be classified into a cluster of orthologous groups or COG by using the COGNITOR program available at the above web site, or by direct BLASTP comparison of the gene of interest to the members of the COGs and analysis of these results as described by Tatusov, R.L., Galperin, M.Y., Natale, D. A. and Koonin, E.V. (2000) The COG database: a tool for genome- scale analysis of protein functions and evolution. Nucleic Acids Research v. 28 n. 1, pp33-36.

The term "homologous coding nucleic acid" also includes nucleic acids comprising nucleotide sequences which encode polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide comprising the amino acid sequence of one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 or to a polypeptide whose expression is inhibited by a nucleic acid comprising a

nucleotide sequence of one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof as determined using the FASTA version 3.0t78 algorithm with the default parameters. Alternatively, protein identity or similarity may be identified
5 using BLASTP with the default parameters, BLASTX with the default parameters, TBLASTN with the default parameters, or tBLASTX with the default parameters. (Altschul, S.F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, Nucleic Acid Res. 25: 3389-3402 (1997)).

The term "homologous coding nucleic acid" also includes coding nucleic acids
10 which hybridize under stringent conditions to a nucleic acid selected from the group consisting of the nucleotide sequences complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and coding nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of the
15 sequences complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105.

As used herein, "stringent conditions" means hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C. Other exemplary stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C, 48°C, 55°C, and 60°C as appropriate
20 for the 5 particular probe being used.

The term "homologous coding nucleic acid" also includes coding nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleotide sequence selected from the group consisting of the sequences complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and coding nucleic
25 acids comprising nucleotide sequences which hybridize under moderate conditions to a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of the sequences complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105. As used herein, "moderate conditions" means hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2xSSC/0.1% SDS at about 42-
30 65°C.

The term "homologous coding nucleic acids" also includes nucleic acids comprising nucleotide sequences which encode a gene product whose activity may

be complemented by a gene encoding a gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105. In some embodiments, the homologous coding nucleic acids may encode a gene product whose activity is complemented by the gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting Seq ID Nos.: 1 to Seq. ID Nos.: 105. In other embodiments, the homologous coding nucleic acids may comprise a nucleotide sequence encodes a gene product whose activity is complemented by one of the polypeptides of Seq ID Nos.: 1 to Seq. ID Nos.: 105 .

The term "homologous antisense nucleic acid" includes nucleic acids comprising a nucleotide sequence having at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of one of the sequences of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and fragments comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides thereof. Homologous antisense nucleic acids may also comprising nucleotide sequences which have at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of the sequences complementary to one of sequences of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and fragments comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides thereof.

Nucleic acid identity may be determined as described above.

The term "homologous antisense nucleic acid" also includes antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleotide sequence complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of the sequence complementary to one Seq ID Nos.: 1 to Seq. ID Nos.: 105. Homologous antisense nucleic acids also include antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleotide sequence selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105, and antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to

a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of one of Seq ID Nos.: 1 to Seq. ID Nos.: 105.

The term "homologous antisense nucleic acid" also includes antisense
5 nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleotide sequence complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and antisense nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of the
10 sequence complementary to one of Seq ID Nos.: 1 to Seq. ID Nos.: 105. Homologous antisense nucleic acids also include antisense nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleotide sequence selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105 and antisense nucleic acids which comprising nucleotide sequences hybridize under
15 moderate conditions to a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of one of Seq ID Nos.: 1 to Seq. ID Nos.: 105.

By "homologous polypeptide" is meant a polypeptide homologous to a polypeptide whose activity or level is inhibited by a nucleic acid comprising a
20 nucleotide sequence selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105 by a homologous antisense nucleic acid. The term "homologous polypeptide" includes polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide whose activity or level is
25 inhibited by a nucleic acid selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105 or by a homologous antisense nucleic acid, or polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide to a fragment comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75,
30 100, or 150 consecutive amino acids of a polypeptide whose activity or level is inhibited by a nucleic acid selected from the group consisting of Seq ID Nos.: 1 to Seq. ID Nos.: 105 or by a homologous antisense nucleic acid. Identity or similarity may be determined using the FASTA version 3. Ot78 algorithm with the default

parameters. Alternatively, protein identity or similarity may be identified using BLASTP with the default parameters, BLASTX with the default parameters, or TBLASTN with the default parameters. (Altschul, S.F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, Nucleic Acid Res. 25: 3389-3402 (1997).
5

The term homologous polypeptide also includes polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide selected from the group consisting of Seq ID Nos.: 2 to Seq. ID Nos.: 106 and polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a fragment comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of a polypeptide selected from the group consisting of Seq ID Nos.: 2 to Seq. ID Nos.: 106.
10

The invention also includes polynucleotides, preferably DNA molecules, that hybridize to one of the nucleic acids of Seq ID Nos.: 2 to Seq. ID Nos.: 106 or the complements of any of the preceding nucleic acids. Such hybridization may be under stringent or moderate conditions as defined above or under other conditions which permit specific hybridization. The nucleic acid molecules of the invention that hybridize to these DNA sequences include oligodeoxynucleotides ("oligos") which hybridize to the target gene under highly stringent or stringent conditions. In general, for oligos between 14 and 70 nucleotides in length the melting temperature (T_m) is calculated using the formula:
15

$$T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}] + 0.41 (\% \text{ G+C}) - (500/N))$$
where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation:
25

$$T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}] + 0.41 (\% \text{ G+C}) - (0.61 (\% \text{ formamide}) - (500/N)))$$
where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below T_m (for DNA-DNA hybrids) or about 10-15 degrees below T_m (for RNA-DNA hybrids).
30

Other hybridization conditions are apparent to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, Current Protocols in Molecular Biology,

Vol. 1, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York, at pp. 6.3.1-6.3.6 and 2.10.3.

By "identifying a compound" is meant to screen one or more compounds in a collection of compounds such as a combinatorial chemical library or other library of chemical compounds or to characterize a single compound by testing the compound
5 in a given assay and determining whether it exhibits the desired activity.

By "inducer" is meant an agent or solution which, when placed in contact with a cell or microorganism, increases transcription, or inhibitor and/or promoter clearance/fidelity, from a desired promoter.

10 As used herein, "nucleic acid" means DNA, RNA, or modified nucleic acids. Thus, the terminology "the nucleic acid of SEQ ID NO: V or "the nucleic acid comprising the nucleotide sequence" includes both the DNA sequence of SEQ ID NO: X and an RNA sequence in which the thymidines in the DNA sequence have been substituted with uridines in the RNA sequence and in which the deoxyribose
15 backbone of the DNA sequence has been substituted with a ribose backbone in the RNA sequence. Modified nucleic acids are nucleic acids having nucleotides or structures which do not occur in nature, such as nucleic acids in which the internucleotide phosphate residues with methylphosphonates, phosphorothioates, phosphoramidates, and phosphate esters. Nonphosphate internucleotide analogs
20 such as siloxane bridges, carbonate bridges, thioester bridges, as well as many others known in the art may also be used in modified nucleic acids. Modified nucleic acids may also comprise, (x-anomeric nucleotide units and modified nucleotides such as 1,2-dideoxy-d-ribofuranose, 1,2-dideoxy-1-phenylribofuranose, and N⁴, N⁴-ethano-5-methyl-cytosine are contemplated for use in the present invention.
25 Modified nucleic acids may also be peptide nucleic acids in which the entire deoxyribose-phosphate backbone has been exchanged with a chemically completely different, but structurally homologous, polyamide (peptide) backbone containing 2-aminoethyl glycogen units.

As used herein, "sub-lethal" means a concentration of an agent below the
30 concentration required to inhibit all cell growth.

A proliferation-required gene or gene family is one where, in the absence or substantial reduction of a gene transcript and/or gene product, growth or viability of the cell or microorganism is reduced or eliminated. Thus, as used herein, the

terminology "proliferation- required" or "required for proliferation" encompasses instances where the absence or substantial reduction of a gene transcript and/or gene product completely eliminates cell growth as well as instances where the absence of a gene transcript and/or gene product merely reduces cell growth. These proliferation-required genes can be used as potential targets for the generation of new antimicrobial agents. To achieve that goal, the present invention also encompasses assays for analyzing proliferation- required genes and for identifying compounds which interact with the gene and/or gene products of the proliferation-required genes. In addition, the present invention contemplates the expression of genes and the purification of the proteins encoded by the nucleic acid sequences identified as required proliferation genes and reported herein. The purified proteins can be used to generate reagents and screen small molecule libraries or other candidate compound libraries for compounds that can be further developed to yield novel antimicrobial compounds.

The invention described herein addresses the need for identifying *Alloiococcus otitidis* proliferation-required gene or gene family that may be used to identify compounds, which are effective in preventing or treating most or all of the disease caused by *Alloiococcus otitidis*. The invention further addresses the need for methods of diagnosing *Alloiococcus otitidis* infection using the genes and the polypeptides identified herein. The inventors have identified novel *Alloiococcus otitidis* open reading frames (Ors), which encode proteins/polypeptides that are essential for the growth and proliferation of the bacteria. More particularly, the newly identified Ors encode polypeptides that are essential for proliferation of *Alloiococcus otitidis*, and thus serve as potential targets for antimicrobial compounds. Thus, in certain embodiments, the invention comprises *Alloiococcus otitidis* Ors encoding polypeptides that are essential for cellular proliferation, transcription gene products of *Alloiococcus otitidis* Ors, including, but not limited to mRNA, antisense RNA, antisense oligonucleotides, and ribozyme molecules, which can be used to inhibit or control growth of the microorganism. The invention relates also to methods of detecting *Alloiococcus otitidis* nucleic acids or polypeptides and kits for diagnosing *Alloiococcus otitidis* infection. The invention also relates to pharmaceutical compositions, in particular antimicrobial compounds in pharmaceutical compositions, for the prevention and/or treatment of bacterial infection, in particular infection

caused by or exacerbated by *Alloiococcus otitidis*.

**B. ALLOIOCOCCUS OTITIDIS ORF POLYNUCLEOTIDES ENCODING POLYPEPTIDES
ESSENTIAL FOR PROLIFERATION**

5

Isolated and purified *Alloiococcus otitidis* ORF polynucleotides of the present invention are contemplated for use in the production of *Alloiococcus otitidis* polypeptides. More specifically, in certain embodiments, the ORFs encode *Alloiococcus otitidis* polypeptides that are essential for cell proliferation. Thus, in one aspect, the present invention provides isolated and purified polynucleotides (ORFs) that encode *Alloiococcus otitidis* essential for cell proliferation. In particular embodiments, a polynucleotide of the present invention is a DNA molecule, wherein the DNA may be genomic DNA, plasmid DNA or cDNA. In a preferred embodiment, a polynucleotide of the present invention is a recombinant polynucleotide, which encodes an *Alloiococcus otitidis* polypeptide comprising an amino acid sequence that has at least 25% identity to an amino acid sequence of one of even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106 or a fragment thereof. In another embodiment, an isolated and purified ORF polynucleotide comprises a nucleotide sequence that has at least 70% identity to one of the ORF polynucleotide nucleotide sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105, a degenerate variant thereof, or a complement thereof. In yet another embodiment, an ORF polynucleotide of one of SEQ ID NO: 1 through SEQ ID NO: 105 is comprised in a plasmid vector and expressed in a host cell. In a preferred embodiment, the host cell is a prokaryotic host cell.

25

As used herein, the term "polynucleotide" means a sequence of nucleotides connected by phosphodiester linkages. Polynucleotides are presented herein in the direction from the 5' to the 3' direction. A polynucleotide of the present invention can comprise from about 10 to about several hundred thousand base pairs. Preferably, a polynucleotide comprises from about 10 to about 3,000 base pairs. Preferred lengths of particular polynucleotide are set forth hereinafter.

30

A polynucleotide of the present invention can be a deoxyribonucleic acid (DNA) molecule, a ribonucleic acid (RNA) molecule, or analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. Where a

polynucleotide is a DNA molecule, that molecule can be a gene, a cDNA molecule or a genomic DNA molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T) and cytosine (C).

“Isolated” means altered “by the hand of man” from the natural state. An
5 “isolated” composition or substance is one that has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not “isolated,” but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is “isolated,” as the term is employed herein.

10 Preferably, an “isolated” polynucleotide is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated *Alloiococcus otitidis* nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of
15 nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. However, the *Alloiococcus otitidis* nucleic acid molecule can also be fused to heterologous protein encoding or regulatory sequences and still be considered isolated.

ORF polynucleotides of the present invention may also be obtained using
20 standard cloning and screening techniques from a cDNA library derived from mRNA. Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries (*e.g.*, an *Alloiococcus otitidis* library) or can be synthesized using well-known and commercially available techniques. As contemplated in the present invention, ORF polynucleotides are obtained using *Alloiococcus otitidis*
25 chromosomal DNA as the template.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences set forth in the odd numbered sequences listed in ID NO: 1 through SEQ ID NO: 105 (and fragments thereof) due to degeneracy of the genetic code, and thus encode the same *Alloiococcus otitidis* polypeptides as those encoded
30 by the amino acid sequences shown in even numbered sequences set forth in SEQ ID NO:2 through SEQ ID NO: 106

Orthologs and allelic variants of the *Alloiococcus otitidis* polynucleotides are readily identified using methods well known in the art. An allelic variant or an

orthologue of the polynucleotides comprises a nucleotide sequence that is typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more homologous to the nucleotide sequence shown in one of the odd numbered sequences set forth in SEQ ID NO:1 through SEQ ID NO: 105, or a
5 fragment of these nucleotide sequences. Such nucleic acid molecules are readily identified as being able to hybridize, preferably under stringent conditions, to the nucleotide sequence shown in one of the odd numbered sequences set forth in SEQ ID NO:1 through SEQ ID NO: 105, or a fragment of these nucleotide sequences.

Moreover, the polynucleotides of the invention can comprise only a fragment
10 of the coding region of an *Alloiococcus otitidis* polynucleotide or gene, such as a fragment of one of the odd numbered sequences set forth in SEQ ID NO:1 through SEQ ID NO: 105.

When the ORF polynucleotides of the invention are used for the recombinant production of *Alloiococcus otitidis* polypeptides of the present invention, the
15 polynucleotide may include the coding sequence for the mature polypeptide, by itself, or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be linked to the
20 coding sequence (see Gentz *et al.*, 1989, incorporated herein by reference). Thus, contemplated in the present invention is the preparation of polynucleotides encoding fusion polypeptides permitting His-tag purification of expression products. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals.

Thus, a polynucleotide encoding a polypeptide of the present invention,
25 including homologs and orthologs from species other than *Alloiococcus otitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of one of the odd numbered sequences set forth in SEQ ID NO:1 through
30 SEQ ID NO: 105 or a fragment thereof; and isolating full-length cDNA and genomic clones containing the polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for

the polypeptide is cut short at the 5' end of the cDNA. This is a consequence of reverse transcriptase, an enzyme with inherently low "processivity" (a measure of the ability of the enzyme to remain attached to the template during the polymerization reaction), failing to complete a DNA copy of the mRNA template during the first-strand cDNA synthesis.

Thus, in certain embodiments, the polynucleotide sequence information provided by the present invention allows for the preparation of relatively short DNA (or RNA) oligonucleotide sequences having the ability to specifically hybridize to gene sequences of the selected polynucleotides disclosed herein. The term "oligonucleotide" as used herein is defined as a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, usually more than three (3), and typically more than ten (10) and up to one hundred (100) or more (although preferably between twenty and thirty). The exact size will depend on many factors, which in turn depends on the ultimate function or use of the oligonucleotide. Thus, in particular embodiments of the invention, nucleic acid probes of an appropriate length are prepared based on a consideration of a selected nucleotide sequence, *e.g.*, a sequence such as that shown in one of the odd numbered sequences set forth in SEQ ID NO:1 through SEQ ID NO: 105. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding an *Alloiococcus otitidis* polypeptide lends them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. These primers are generated in any manner, including chemical synthesis, DNA replication, reverse transcription, or a combination thereof. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of an ORF polynucleotide that encodes an *Alloiococcus otitidis* polypeptide from prokaryotic cells using polymerase chain reaction (PCR) technology.

In certain embodiments, it is advantageous to employ a polynucleotide of the present invention in combination with an appropriate label for detecting hybrid formation. A wide variety of appropriate labels are known in the art, including

radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal.

Polynucleotides which are identical or sufficiently identical to a nucleotide sequence contained in one of the odd numbered sequences set forth in SEQ ID NO:1 through SEQ ID NO: 105, or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification (PCR) reaction, to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than *Alloiococcus otitidis*) that have a high sequence similarity to polynucleotide sequences set forth in one of the odd numbered sequences set forth in SEQ ID NO:1 through SEQ ID NO:105, or a fragment thereof. Typically these nucleotide sequences are from at least 70% identical to at least about 95% identical to that of the reference polynucleotide sequence. The probes or primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides.

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, Frohman *et al.*, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology [Promega, Madison, WI], for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an "adaptor" sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction are then analyzed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA to give a complete

sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes probe molecules that are complementary to at least a 10 to about 70 nucleotides long stretch of a polynucleotide that encodes an *Alloiococcus otitidis* polypeptide, such as that shown in one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106. A size of at least 10 nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. It is generally preferable to design nucleic acid molecules with gene-complementary stretches of 25 to 40 nucleotides, 55 to 70 nucleotides, or even longer where desired. For example, such fragments are readily prepared by directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology (U.S. Patent 4,683,202, incorporated herein by reference), or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction enzyme sites.

In another aspect, the present invention contemplates an isolated and purified polynucleotide comprising a nucleotide sequence that is identical or complementary to a segment of at least 10 contiguous bases of one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105, wherein the polynucleotide hybridizes to a polynucleotide that encodes an *Alloiococcus otitidis* polypeptide. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105. For example, the polynucleotide of the invention can comprise a segment of bases identical or complementary to from 40 to 55 contiguous bases of the disclosed nucleotide sequences.

Accordingly, a polynucleotide probe molecule of the invention can be used for its ability to selectively form duplex molecules with complementary stretches of the

gene. Depending on the application envisioned, varying conditions of hybridization are employed to achieve varying degrees of selectivity of the probe toward the target sequence. For applications requiring a high degree of selectivity, relatively stringent conditions are employed to form the hybrids. Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate an *Alloicoccus otitidis* homologous polypeptide coding sequence from other cells, functional equivalents, or the like, less stringent hybridization conditions are typically needed to allow formation of the heteroduplex (see Table 2). Cross-hybridizing species are thereby readily identified as positively hybridizing signals with respect to control hybridizations. Thus, hybridization conditions are readily manipulated, and thus will generally be a method of choice depending on the desired results.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate a homologous polypeptide coding sequence from other cells, functional equivalents, or the like, less stringent hybridization conditions are typically needed to allow formation of the heteroduplex. Cross-hybridizing species are thereby readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions are readily manipulated, and thus are generally a method of choice depending on the desired results.

The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

TABLE 2
STRINGENCY CONDITIONS

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp) ¹	Hybridization Temperature and Buffer ^H	Wash Temperature and Buffer ^H
A	DNA:DNA	> 50	65°C; 1xSSC -or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
B	DNA:DNA	< 50	T _B ; 1xSSC	T _B ; 1xSSC
C	DNA:RNA	> 50	67°C; 1xSSC -or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
D	DNA:RNA	< 50	T _D ; 1xSSC	T _D ; 1xSSC
E	RNA:RNA	> 50	70°C; 1xSSC -or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
F	RNA:RNA	< 50	T _F ; 1xSSC	T _F ; 1xSSC
G	DNA:DNA	> 50	65°C; 4xSSC -or- 42°C; 4xSSC, 50% formamide	65°C; 1xSSC
H	DNA:DNA	< 50	T _H ; 4xSSC	T _H ; 4xSSC
I	DNA:RNA	> 50	67°C; 4xSSC -or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
J	DNA:RNA	< 50	T _J ; 4xSSC	T _J ; 4xSSC
K	RNA:RNA	> 50	70°C; 4xSSC -or- 50EC; 4xSSC, 50% formamide	67°C; 1xSSC
L	RNA:RNA	< 50	T _L ; 2xSSC	T _L ; 2xSSC
M	DNA:DNA	> 50	50°C; 4xSSC -or- 40°C; 6xSSC, 50% formamide	50°C; 2xSSC
N	DNA:DNA	< 50	T _N ; 6xSSC	T _N ; 6xSSC
O	DNA:RNA	> 50	55°C; 4xSSC -or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
P	DNA:RNA	< 50	T _P ; 6xSSC	T _P ; 6xSSC
Q	RNA:RNA	> 50	60°C; 4xSSC -or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
R	RNA:RNA	< 50	T _R ; 4xSSC	T _R ; 4xSSC

(bp)¹: The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target

polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence

5 complementarity.

Buffer^H: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4), can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

10 T_B through T_R: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(EC) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(EC) = 81.5 +
15 16.6(log₁₀[Na⁺]) + 0.41(%G+C) - (600/N), where N is the number of bases in the hybrid, and [Na⁺] is the concentration of sodium ions in the hybridization buffer ([Na⁺] for 1xSSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual, Cold
20 Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Ausubel *et al.*, 1995, Current Protocols in Molecular Biology, Eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

In addition to the nucleic acid molecules encoding *Alloiococcus otitidis* polypeptides described above, another aspect of the invention pertains to isolated
25 nucleic acid molecules that are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid
30 can be complementary to an entire *Alloiococcus otitidis* coding strand, or to only a fragment thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an *Alloiococcus otitidis* polypeptide.

The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues, *e.g.*, the entire coding region of each of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding an *Alloiococcus otitidis* polypeptide. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequence encoding the *Alloiococcus otitidis* polypeptides disclosed herein antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of *Alloiococcus otitidis* mRNA, but more preferably is an oligonucleotide which is antisense to only a fragment of the coding or noncoding region of *Alloiococcus otitidis* mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of *Alloiococcus otitidis* mRNA.

An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-

methythio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an *Alloiococcus otitidis* polypeptide to thereby inhibit expression of the polypeptide, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of an antisense nucleic acid molecule of the invention includes direct injection at a tissue site. Alternatively, an antisense nucleic acid molecule can be modified to target selected cells and then administered systemically. For example, for systemic administration, an antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual γ -units, the strands run parallel to each other (Gaultier *et al.*, 1987). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.*, 1987) or a chimeric RNA-DNA analogue (Inoue *et al.*, 1987).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes
5 described in Haselhoff and Gerlach, 1988) can be used to catalytically cleave *Alloiococcus otitidis* mRNA transcripts to thereby inhibit translation of *Alloiococcus otitidis* mRNA. A ribozyme having specificity for an *Alloiococcus otitidis*-encoding nucleic acid can be designed based upon the nucleotide sequence of an *Alloiococcus otitidis* cDNA disclosed herein. For example, a derivative of a
10 Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an *Alloiococcus otitidis*-encoding mRNA. See, e.g., Cech *et al.* U.S. 4,987,071 and Cech *et al.* U.S. 5,116,742 both incorporated herein in their entirety by reference. Alternatively, *Alloiococcus otitidis* mRNA can be used to select a catalytic RNA
15 having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel and Szostak, 1993.

Alternatively *Alloiococcus otitidis* gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the *Alloiococcus otitidis* gene (e.g., the *Alloiococcus otitidis* gene promoter and/or
20 enhancers) to form triple helical structures that prevent transcription of the *Alloiococcus otitidis* gene in target cells. See generally, Helene, 1991; Helene *et al.*, 1992; and Maher, 1992.

Alloiococcus otitidis gene expression can also be inhibited using RNA interference (RNAi). This is a technique for post-transcriptional gene silencing
25 (PTGS), in which target gene activity is specifically abolished with cognate double-stranded RNA (dsRNA). RNAi resembles in many aspects PTGS in plants and has been detected in many invertebrates including trypanosome, hydra, planaria, nematode and fruit fly (*Drosophila melanogaster*). It may be involved in the modulation of transposable element mobilization and antiviral state formation. RNAi
30 in mammalian systems is disclosed in WO 00/63364, which is incorporated by reference herein in its entirety. Basically, dsRNA of at least about 600 nucleotides, homologous to the target is introduced into the cell and a sequence specific reduction in gene activity is observed.

C. ALLOIOCOCCUS OTITIDIS POLYPEPTIDES

In particular embodiments, the present invention provides isolated and
5 purified *Alloiococcus otitidis* polypeptides. Preferably, an *Alloiococcus otitidis*
polypeptide of the invention is a recombinant polypeptide. In certain embodiments,
an *Alloiococcus otitidis* polypeptide of the present invention comprises the amino acid
sequence that has at least 25% identity to the amino acid sequence of one of the
even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106, a
10 biological equivalent thereof, or a fragment thereof.

An *Alloiococcus otitidis* polypeptide according to the present invention
encompasses a polypeptide that comprises: 1) the amino acid sequence shown in
one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO:
106) functional and non-functional naturally occurring variants or biological
15 equivalents of *Alloiococcus otitidis* polypeptides of the even numbered sequences set
forth in SEQ ID NO: 2 through SEQ ID NO: 106 and recombinantly produced variants
or biological equivalents of *Alloiococcus otitidis* polypeptides set out in SEQ ID NO: 2
through SEQ ID NO: 106) polypeptides isolated from organisms other than
Alloiococcus otitidis (orthologs of *Alloiococcus otitidis* polypeptides.)

20 A biological equivalent or variant of an *Alloiococcus otitidis* polypeptide
according to the present invention encompasses 1) a polypeptide isolated from
Alloiococcus otitidis; and 2) a polypeptide that contains substantial homology to an
Alloiococcus otitidis polypeptide.

Biological equivalents or variants of *Alloiococcus otitidis* include both
25 functional and non-functional *Alloiococcus otitidis* polypeptides. Functional biological
equivalents or variants are naturally occurring amino acid sequence variants of an
Alloiococcus otitidis polypeptide that maintain the ability to elicit an immunological or
antigenic response in a subject. Functional variants will typically contain only
conservative substitutions of one or more amino acids in any one of even numbered
30 sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106 or substitution,
deletion or insertion of non-critical residues in non-critical regions of the polypeptide.

The present invention further provides non-*Alloiococcus otitidis* orthologues of
Alloiococcus otitidis polypeptides. Orthologues of *Alloiococcus otitidis* polypeptides

are polypeptides that are isolated from non-*Alloiococcus otitidis* organisms and possess antigenic capabilities of the *Alloiococcus otitidis* polypeptide. Orthologues of an *Alloiococcus otitidis* polypeptide can readily be identified as comprising an amino acid sequence that is substantially homologous to one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106.

Modifications and changes can be made in the structure of a polypeptide of the present invention and still obtain a molecule having *Alloiococcus otitidis* antigenicity. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of antigenicity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like properties.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art (Kyte & Doolittle, 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid residue determines the secondary and tertiary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within +/-2 is

preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biologically functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. U.S. Pat. No. 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.* with a biological property of the polypeptide.

As detailed in U.S. Pat. No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine (See Table 3, below). The present invention thus contemplates functional or biological equivalents of an *Allōiococcus otitidis* polypeptide as set forth above.

TABLE 3:
AMINO ACID SUBSTITUTIONS

Original Residue	Exemplary Residue Substitution
Ala	Gly; Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg
Met	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Biological or functional equivalents of a polypeptide are also prepared using
 5 site-specific mutagenesis. Site-specific mutagenesis is a technique useful in the
 preparation of second generation polypeptides, or biologically functional equivalent
 polypeptides or peptides, derived from the sequences thereof, through specific
 mutagenesis of the underlying DNA. As noted above, such changes can be
 desirable where amino acid substitutions are desirable. The technique further
 10 provides a capacity to prepare and test sequence variants, for example, incorporating
 one or more of the foregoing considerations, by introducing one or more nucleotide
 sequence changes into the DNA. Site-specific mutagenesis allows the production of
 mutants through the use of specific oligonucleotide sequences which encode the
 DNA sequence of the desired mutation, as well as a sufficient number of adjacent
 15 nucleotides, to provide a primer sequence of sufficient size and sequence complexity
 to form a stable duplex on both sides of the deletion junction being traversed.
 Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to
 10 residues on both sides of the site of the alteration of the sequence.

In general, the technique of site-specific mutagenesis is well known in the art.
 20 As will be appreciated, the technique typically employs a phage vector, that can exist

in both a single stranded and double stranded form. Typically, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector which includes within its sequence a DNA sequence which encodes all or a portion of the *Alloicoccus otitidis* polypeptide sequence selected. An
5 oligonucleotide primer bearing the desired mutated sequence is prepared (*e.g.*, synthetically). This primer is then annealed to the singled-stranded vector, and extended by the use of enzymes such as *Escherichia coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated
10 sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells such as *Escherichia coli* cells and clones are selected which include recombinant vectors bearing the mutation. Commercially available kits come with all the reagents necessary, except the oligonucleotide primers.

15 An *Alloicoccus otitidis* polypeptide or polypeptide antigen of the present invention is understood to be any *Alloicoccus otitidis* polypeptide comprising substantial sequence similarity, structural similarity and/or functional similarity to an *Alloicoccus otitidis* polypeptide comprising the amino acid sequence of one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106. In
20 addition, an *Alloicoccus otitidis* polypeptide or polypeptide antigen of the invention is not limited to a particular source. Thus, the invention provides for the general detection and isolation of the polypeptides from a variety of sources.

It is contemplated in the present invention, that an *Alloicoccus otitidis* polypeptide may advantageously be cleaved into fragments for use in further
25 structural or functional analysis, or in the generation of reagents such as *Alloicoccus otitidis*-related polypeptides and *Alloicoccus otitidis*-specific antibodies. This can be accomplished by treating purified or unpurified *Alloicoccus otitidis* polypeptides with a peptidase such as endoproteinase glu-C (Boehringer, Indianapolis, IN). Treatment with CNBr is another method by which peptide fragments may be produced from
30 natural *Alloicoccus otitidis* polypeptides. Recombinant techniques also can be used to produce specific fragments of an *Alloicoccus otitidis* polypeptide.

In addition, the inventors also contemplate that compounds sterically similar to a particular *Alloicoccus otitidis* polypeptide antigen, called peptidomimetics, may

be formulated to mimic the key portions of the peptide structure. Peptidemimetics are peptide-containing molecules that mimic elements of protein secondary structure. (See, for example, Johnson *et al.*, 1993.) The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of receptor and ligand.

Successful applications of the peptide mimetic concept have thus far focused on mimetics of β -turns within proteins. Likely β -turn structures, within *Alloiococcus otitidis*, can be predicted by computer-based algorithms as discussed above. Once the component amino acids of the turn are determined, mimetics can be constructed to achieve a similar spatial orientation of the essential elements of the amino acid side chains, as discussed in Johnson *et al.*, 1993.

Fragments of the *Alloiococcus otitidis* polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as a part, but not all, of the amino acid sequence. The fragment can comprise, for example, at least 7 or more (e.g., 8, 10, 12, 14, 16, 18, 20 or more) contiguous amino acids of an amino acid sequence selected from one of the even numbered sequences set forth in SEQ ID NO.: 2 through SEQ ID NO.: 106. Fragments may be "freestanding" or comprised within a larger polypeptide of which they form a part or region, most preferably as a single, continuous region. In one embodiment, the fragments include at least one epitope of the mature polypeptide sequence.

"Fusion protein" refers to a protein encoded by two, often unrelated, fused genes or fragments thereof. For example, fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof have been described. In many cases, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties (see, e.g., EP-A 0232 2621). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

D. ALLOIOCOCCUS OTITIDIS POLYNUCLEOTIDE AND POLYPEPTIDE VARIANTS

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring variant such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match

between the sequences tested. Methods to determine identity are codified in publicly available computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., *et al* 1984), BLASTP, BLASTN, and FASTA (Altschul, S. F., *et al.*, 1990. The BLASTX program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., *et al.*, 1990). The well known Smith-Waterman algorithm may also be used to determine identity.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of one of SEQ ID NO:1 through SEQ ID NO: 105, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105 by the numerical percent of the respective percent identity (divided by 100) and subtracting that product from said total number of nucleotides in one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105.

For example, the alterations in an isolated *Alloiococcus otitidis* polynucleotide comprise a polynucleotide sequence that has at least 70% identity to the nucleic acid sequence of one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105; a degenerate variant thereof or a fragment thereof, wherein the polynucleotide sequence may include up to n_n nucleic acid alterations over the entire polynucleotide region of the nucleic acid sequence of any one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105, wherein n_n is the maximum number of alterations and is calculated by the formula:

$$n_n \leq x_n - (x_n \cdot y),$$

in which x_n is the total number of nucleic acids of one of SEQ ID NO:1 through SEQ ID NO:105 and y has a value of 0.70, wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting such product from x_n . Of course, y may also have a value of 0.80 for 80%, 0.85 for 85%, 0.90 for 90% 0.95 for 95%, etc.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of any one of even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106, that is 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percentage identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106, or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in one of SEQ ID NO: 2 through SEQ ID NO: 106, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of $x_{\text{sub.a}}$ and y is rounded down to the nearest integer prior to subtracting it from x_a .

E. VECTORS, HOST CELLS AND RECOMBINANT *ALLOIOCOCCUS OTITIDIS* POLYPEPTIDES

In a preferred embodiment, the present invention provides expression vectors comprising ORF polynucleotides that encode *Alloiococcus otitidis* polypeptides. Preferably, the expression vectors of the present invention comprise ORF

polynucleotides that encode *Alloiococcus otitidis* polypeptides comprising the amino acid residue sequence of one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106. More preferably, the expression vectors of the present invention comprise a polynucleotide comprising the nucleotide base sequence of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105. Even more preferably, the expression vectors of the invention comprise a polynucleotide operatively linked to promoter. Still more preferably, the expression vectors of the invention comprise a polynucleotide operatively linked to a prokaryotic promoter. Alternatively, the expression vectors of the present invention comprise a polynucleotide operatively linked to an enhancer-promoter, that is, an eukaryotic promoter. The expression vectors further comprise a polyadenylation signal that is positioned 3' of the carboxy-terminal amino acid and within a transcriptional unit of the encoded polypeptide.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase.

Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

In one embodiment, the coding sequence of the *Alloiococcus otitidis* polynucleotide is cloned into a pGEX expression vector to create a vector encoding a fusion protein comprising, from the N-terminus to the C-terminus, GST-thrombin cleavage site-*Alloiococcus otitidis* polypeptide. The fusion protein can be purified by

affinity chromatography using glutathione-agarose resin. Recombinant *Alloiococcus otitidis* polypeptide unfused to GST can be recovered by cleavage of the fusion protein with thrombin.

Examples of suitable inducible non-fusion *Escherichia coli* expression vectors include pTrc (Amann *et al.*, 1988) and pET 11 d (Studier *et al.*, 1990). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11 d vector relies on transcription from a T7 gn1 0-lac fusion promoter mediated by a coexpressed viral RNA polymerase T7 gnl. This viral polymerase is supplied by host strains BL21 (DE3) or HMS 174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *Escherichia coli* is to express the protein in a host bacterium with an impaired capacity to proteolytically cleave the recombinant protein. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *Escherichia coli*. Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA mutagenesis or synthesis techniques.

In another embodiment, the *Alloiococcus otitidis* polynucleotide expression vector is a yeast expression vector. Examples of vectors for expression in a yeast such as *S. cerevisiae* include pYepSec I (Baldari, *et al.*, 1987), pMFa (Kurjan and Herskowitz, 1982), pJRY88 (Schultz *et al.*, 1987), and pYES2 (Invitrogen Corporation, San Diego, CA).

Alternatively, an *Alloiococcus otitidis* polynucleotide is expressed in insect cells using, for example, baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 or Sf 21 cells) include the pAc series (Smith *et al.*, 1983) and the pVL series (Lucklow and Summers, 1989).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987) and pMT2PC (Kaufman *et al.*, 1987). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements.

As used herein, a promoter is a region of a DNA molecule typically within about 100 nucleotide pairs in front of (upstream of) the point at which transcription begins (*i.e.*, a transcription start site). That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes. As used herein, the term "promoter" includes what is referred to in the art as an upstream promoter region, a promoter region or a promoter of a generalized eukaryotic RNA Polymerase II transcription unit.

Another type of discrete transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and expression level for a particular encoding region (*e.g.*, gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to that enhancer. Unlike a promoter, an enhancer can function when located at variable distances from transcription start sites so long as a promoter is present.

As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer-promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" means that an enhancer-promoter is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that enhancer-promoter. Means for operatively linking an enhancer-promoter to a coding sequence are well known in the art. As is also well known in the art, the precise orientation and location relative to a coding sequence whose transcription is controlled, is dependent *inter alia* upon the specific nature of the enhancer-promoter. Thus, a TATA box minimal promoter is typically located from about 25 to about 30 base pairs upstream of a transcription initiation site and an upstream promoter element is typically located from about 100 to about 200 base pairs upstream of a transcription initiation site. In contrast, an enhancer can be located downstream from the initiation site and can be at a considerable distance from that site.

An enhancer-promoter used in a vector construct of the present invention can be any enhancer-promoter that drives expression in a cell to be transfected. By employing an enhancer-promoter with well-known properties, the level and pattern of gene product expression can be optimized.

For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus (CMV) and Simian Virus 40 (SV40). For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual" 2nd, ed, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, incorporated herein by reference.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.*, 1987), lymphoid-specific promoters (Calame and Eaton, 1988), in particular promoters of T cell receptors (Winoto and Baltimore, 1989) and immunoglobulins (Banerji *et al.*, 1983), Queen and Baltimore (1983), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989), pancreas-specific promoters (Edlund *et al.*, 1985), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. 4, 873,316 and EP 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss, 1990) and the α -fetoprotein promoter (Campes and Tilghman, 1989).

The invention further provides a recombinant expression vector comprising a DNA molecule encoding an *Alloiococcus otitidis* polypeptide cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to *Alloiococcus otitidis* mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic or eukaryotic cell. For example, an *Alloiococcus otitidis* polypeptide can be expressed in bacterial cells such as *Escherichia coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO), NIH3T3, PER C6, NSO, VERO or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA is can be introduced into prokaryotic or eukaryotic cells via conventional transformation, infection or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, protoplast fusion, direct microinfection. Another recognized technique for introducing DNA into a host cell is "infection", such as by adenovirus infection or electroporation. Suitable methods for transforming, infecting or transfecting host cells can be found in Sambrook, *et al.* ("Molecular Cloning: A Laboratory Manual" 2nd ed, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

The most widely used method is transfection mediated by either calcium phosphate or DEAE-dextran. Although the mechanism remains unclear, it is believed that the transfected DNA enters the cytoplasm of the cell by endocytosis and is transported to the nucleus. Depending on the cell type, up to 90% of a population of cultured cells can be transfected at any one time. Because of its high efficiency, transfection mediated by calcium phosphate or DEAE-dextran is the method of choice for experiments that require transient expression of the foreign DNA in large numbers of cells. Calcium phosphate-mediated transfection is also used to establish cell lines that integrate copies of the foreign DNA, which are usually arranged in head-to-tail tandem arrays into the host cell genome.

In the protoplast fusion method, protoplasts derived from bacteria carrying high numbers of copies of plasmid of interest are mixed directly with cultured mammalian cells. After fusion of the cell membranes (usually with polyethylene glycol), the contents of the bacteria are delivered into the cytoplasm of the mammalian cells and the plasmid DNA is transported to the nucleus. Protoplast fusion is not as efficient as transfection for many of the cell lines that are commonly used for transient expression assays, but it is useful for cell lines in which endocytosis of DNA occurs inefficiently. Protoplast fusion frequently yields multiple copies of the plasmid DNA tandemly integrated into the host chromosome.

The application of brief, high-voltage electric pulses (electroporation) to a variety of mammalian and plant cells leads to the formation of nanometer-sized pores in the plasma membrane. DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation can be extremely efficient and can be used both for transient expression of cloned genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast to calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

Liposome transfection involves encapsulation of DNA and RNA within liposomes, followed by fusion of the liposomes with the cell membrane. The mechanism of how DNA is delivered into the cell is unclear, but transfection efficiencies can be as high as 90%.

Direct microinjection of a DNA molecule into nuclei has the advantage of not exposing DNA to cellular compartments such as low-pH endosomes. Microinjection therefore used primarily as a method to establish lines of cells that carry integrated copies of the DNA of interest.

The use of adenovirus as a vector for cell transfection is well known in the art. Adenovirus vector-mediated cell transfection has been reported for various cells (Stratford-Perricaudet, *et al.* 1992).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, is used to produce (*i.e.*, express) an *Alloiococcus otitidis* polypeptide. Accordingly, the invention further provides methods for producing an *Alloiococcus*

otitidis polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding an *Alloiococcus otitidis* polypeptide has been introduced) in a suitable medium until the *Alloiococcus otitidis* polypeptide is produced. In
5 another embodiment, the method further comprises isolating the *Alloiococcus otitidis* polypeptide from the medium or the host cell.

A coding sequence of an expression vector is operatively linked to a transcription-terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences
10 located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA). Transcription-terminating regions are well known in the art. A preferred transcription-terminating region used in
15 an adenovirus vector construct of the present invention comprises a polyadenylation signal of SV40 or the protamine gene.

An expression vector comprises a polynucleotide that encodes an *Alloiococcus otitidis* polypeptide. Such a polypeptide is meant to include a sequence of nucleotide bases encoding an *Alloiococcus otitidis* polypeptide sufficient in length
20 to distinguish the segment from a polynucleotide segment encoding a non-*Alloiococcus otitidis* polypeptide. A polypeptide of the invention can also encode biologically functional polypeptides or peptides which have variant amino acid sequences, such as with changes selected based on considerations such as the relative hydropathic score of the amino acids being exchanged. These variant
25 sequences are those isolated from natural sources or induced in the sequences disclosed herein using a mutagenic procedure such as site-directed mutagenesis.

Preferably, an expression vector of the present invention comprises a polynucleotide that encodes a polypeptide comprising the amino acid residue sequence of one of the even numbered sequences set forth in SEQ ID NO: 2 through
30 SEQ ID NO: 4036. An expression vector can include an *Alloiococcus otitidis* polypeptide coding region itself of any of the *Alloiococcus otitidis* polypeptides noted above or it can contain coding regions bearing selected alterations or modifications in the basic coding region of such an *Alloiococcus otitidis* polypeptide. Alternatively,

such vectors or fragments can also encode larger polypeptides or polypeptides which nevertheless include the basic coding region. In any event, it should be appreciated that due to codon redundancy as well as biological functional equivalence, this aspect of the invention is not limited to the particular DNA molecules corresponding to the polypeptide sequences noted above.

Exemplary vectors include the mammalian expression vectors of the pCMV family including pCMV6b and pCMV6c (Chiron Corp., Emeryville CA.). In certain cases, and specifically in the case of these individual mammalian expression vectors, the resulting constructs can require co-transfection with a vector containing a selectable marker such as pSV2neo. Via co-transfection into a dihydrofolate reductase-deficient Chinese hamster ovary cell line, such as DG44, clones expressing *Alloiococcus otitidis* polypeptides by virtue of DNA incorporated into such expression vectors can be detected.

A DNA molecule of the present invention can be incorporated into a vector by a number of techniques that are well known in the art. For instance, the vector pUC18 has been demonstrated to be of particular value in cloning and expression of genes. Likewise, the related vectors M13mp18 and M13mp19 can also be used in certain embodiments of the invention, in particular, in performing dideoxy sequencing.

An expression vector of the present invention is useful both as a means for preparing quantities of the *Alloiococcus otitidis* polypeptide-encoding DNA itself, and as a means for preparing the encoded polypeptide and peptides. It is contemplated that where *Alloiococcus otitidis* polypeptides of the invention are made by recombinant means, one can employ either prokaryotic or eukaryotic expression vectors as shuttle systems. In another aspect, the recombinant host cells of the present invention are prokaryotic host cells. Preferably, the recombinant host cells of the invention are bacterial cells of the DH5 α strain of *Escherichia coli*. In general, prokaryotes are preferred for the initial cloning of DNA sequences and constructing the vectors useful in the invention. For example, *Escherichia coli* K12 strains can be particularly useful. Other microbial strains that can be used include *Escherichia coli* B, *Escherichia coli* W3110 (ATCC No. 273325) and *Escherichia coli* 1976 (ATCC No. 31537). *Bacilli* such as *Bacillus subtilis*, or other enterobacteriaceae such as *Salmonella typhimurium* or other *Salmonella* species or *Serratia marcesans*, and

various pseudomonas species can be used. These examples are, of course, intended to be illustrative rather than limiting.

In general, plasmid vectors containing replicon and control sequences that are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences that are capable of providing phenotypic selection in transformed cells. For example, *Escherichia coli* can be transformed using pBR322, a plasmid derived from an *Escherichia coli* species (Bolivar, *et al.* 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage, must also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of its own polypeptides.

Those promoters most commonly used in recombinant DNA construction include the β -lactamase (penicillinase) and lactose promoter systems (Chang, *et al.* 1978; Itakura, *et al.* 1977, Goeddel, *et al.* 1979; Goeddel, *et al.* 1980) and a tryptophan (TRP) promoter system (EP 0036776; Siebwenlist *et al.* 1980). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to introduce functional promoters into plasmid vectors (Siebwenlist, *et al.* 1980).

In addition to prokaryotes, eukaryotic microbes such as yeast can also be used. *Saccharomyces cerevisiae* or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used (Stinchcomb, *et al.* 1979; Kingsman, *et al.* 1979; Tschemper, *et al.* 1980). This plasmid already contains the *trp1* gene that provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the *trp1* lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoter sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (PGK) (Hitzeman, *et al.* 1980) or other glycolytic enzymes (Hess, *et al.* 1968; Holland, *et al.* 1978) such as enolase, glyceraldehyde-3-

phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences
5 associated with these genes are also introduced into the expression vector downstream from the sequences to be expressed to provide polyadenylation of the mRNA and termination. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes
10 associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Any plasmid vector containing a yeast-compatible promoter, origin of replication, and termination sequences is suitable.

In addition to microorganisms, cultures of cells derived from multicellular
15 organisms can also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years. Examples of such useful host cell lines are AtT-20, VERO, HeLa, NSO, PER C6, Chinese hamster ovary (CHO) cell lines,
20 W138, BHK, COSM6, COS-7, 293, VERO and MDCK cell lines. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

25 Where expression of recombinant *Alloicoccus otitidis* polypeptides is desired and a eukaryotic host is contemplated, it is most desirable to employ a vector, such as a plasmid, that incorporates a eukaryotic origin of replication. Additionally, for the purposes of expression in eukaryotic systems, one desires to position the *Alloicoccus otitidis* encoding sequence adjacent to and under the control of an
30 effective eukaryotic promoter such as promoters used in combination with Chinese hamster ovary cells (CHO). To bring a coding sequence under control of a promoter, whether it is eukaryotic or prokaryotic, what is generally needed is to position the 5' end of the translation initiation side of the proper translational reading frame of the

polypeptide between about 1 and about 50 nucleotides 3' of or downstream with respect to the promoter chosen. Furthermore, where eukaryotic expression is anticipated, one would typically desire to incorporate an appropriate polyadenylation site into the transcriptional unit that includes the *Alloiococcus otitidis* polypeptide.

5 A transfected cell can be prokaryotic or eukaryotic. Preferably, the host cells of the invention are prokaryotic host cells. Where it is of interest to produce an *Alloiococcus otitidis* polypeptide, cultured prokaryotic host cells are of particular interest.

10 In yet another embodiment, the present invention contemplates a process or method of preparing *Alloiococcus otitidis* polypeptides comprising transfecting, transforming or infecting cells with a polynucleotide that encodes an *Alloiococcus otitidis* polypeptide to produce transformed host cells; and maintaining the transformed host cells under biological conditions sufficient for expression of the polypeptide. Preferably, the transformed host cells are prokaryotic cells.

15 Alternatively, the host cells are eukaryotic cells. More preferably, the prokaryotic cells are bacterial cells of the DH5 α strain of *Escherichia coli*. Even more preferably, the polynucleotide transfected into the transformed cells comprises the nucleic acid sequence of one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105. Additionally, transfection is accomplished using an expression

20 vector disclosed above. A host cell used in the process is capable of expressing a functional, recombinant *Alloiococcus otitidis* polypeptide.

Following transfection, the cell is maintained under culture conditions for a period of time sufficient for expression of an *Alloiococcus otitidis* polypeptide. Culture conditions are well known in the art and include ionic composition and concentration,

25 temperature, pH and the like. Typically, transfected cells are maintained under culture conditions in a culture medium. Suitable media for various cell types are well known in the art. In a preferred embodiment, temperature is from about 20°C to about 50°C, more preferably from about 30°C to about 40°C and, even more preferably about 37°C.

30 The pH is preferably from about a value of 6.0 to a value of about 8.0, more preferably from about a value of about 6.8 to a value of about 7.8 and, most preferably about 7.4. Osmolality is preferably from about 200 milliosmols per liter (mosm/L) to about 400 mosm/L and, more preferably from about 290 mosm/L to

about 310 mosm/L. Other biological conditions needed for transfection and expression of an encoded protein are well known in the art.

Transfected cells are maintained for a period of time sufficient for expression of an *Alloiococcus otitidis* polypeptide. A suitable time depends *inter alia* upon the cell type used and is readily determinable by a skilled artisan. Typically, maintenance time is from about 2 to about 14 days.

Recombinant *Alloiococcus otitidis* polypeptide is recovered or collected either from the transfected cells or the medium in which those cells are cultured. Recovery comprises isolating and purifying the *Alloiococcus otitidis* polypeptide. Isolation and purification techniques for polypeptides are well known in the art and include such procedures as precipitation, filtration, chromatography, electrophoresis and the like.

F. ANTIBODIES IMMUNOREACTIVE WITH *ALLOIOCOCCUS OTITIDIS* POLYPEPTIDES

In still another embodiment, the present invention provides antibodies immunoreactive with *Alloiococcus otitidis* polypeptides. Preferably, the antibodies of the invention are monoclonal antibodies. Additionally, the *Alloiococcus otitidis* polypeptides comprise the amino acid residue sequence of one of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106. Means for preparing and characterizing antibodies are well known in the art (See, e.g., Antibodies "A Laboratory Manual", E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988). Polyclonal antisera is obtained by bleeding an immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is then recovered by centrifugation.

Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogen comprising a polypeptide or polynucleotide of the present invention, and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Typically an animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster or a guinea pig. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

As is well known in the art, a given polypeptide or polynucleotide may vary in its immunogenicity. It is often necessary therefore to couple the immunogen (e.g., a polypeptide or polynucleotide) of the present invention with a carrier. Exemplary and

preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers.

Means for conjugating a polypeptide or a polynucleotide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

As is also well known in the art, immunogenicity to a particular immunogen can be enhanced by the use of non-specific stimulators of the immune response known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant, incomplete Freund's adjuvants, cholera toxin (e.g. mutant cholera toxin E29H; see published International Patent Application WO 00/18434), and aluminum hydroxide adjuvant.

The amount of immunogen used for the production of polyclonal antibodies depends upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies is monitored by sampling blood from the immunized animal at various points following immunization. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored.

In another aspect, the present invention contemplates a process of producing an antibody immunoreactive with an *Alloiococcus otitidis* polypeptide comprising the steps of (a) transfecting recombinant host cells with a polynucleotide that encodes an *Alloiococcus otitidis* polypeptide; (b) culturing the host cells under conditions sufficient for expression of the polypeptide; (c) recovering the polypeptides; and (d) preparing the antibodies to the polypeptides. Preferably, the host cell is transfected with the polynucleotide of one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 4035. Even more preferably, the present invention provides antibodies prepared according to the process described above.

A monoclonal antibody of the present invention can be readily prepared through use of well-known techniques such as those exemplified in U.S. Pat. No. 4,196,265, herein incorporated by reference. Typically, a technique involves first immunizing a suitable animal with a selected antigen (e.g., a polypeptide or

polynucleotide of the present invention) in a manner sufficient to provide an immune response. Rodents such as mice and rats are preferred animals. Spleen cells from the immunized animal are then fused with cells of an immortal myeloma cell. Where the immunized animal is a mouse, a preferred myeloma cell is a murine NS-1 myeloma cell.

The fused spleen/myeloma cells are cultured in a selective medium to select fused spleen/myeloma cells from the parental cells. Fused cells are separated from the mixture of non-fused parental cells, *e.g.*, by the addition of agents that block the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides. Where azaserine is used, the media is supplemented with hypoxanthine.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants for reactivity with an antigen-polypeptide. The selected clones can then be propagated indefinitely to provide the monoclonal antibody.

By way of specific example, to produce an antibody of the present invention, mice are injected intraperitoneally with between about 1-200 μ g of an antigen comprising a polypeptide of the present invention. B lymphocyte cells are stimulated to grow by injecting the antigen in association with an adjuvant such as complete Freund's adjuvant (CFA; a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*). At some time (*e.g.*, at least two weeks) after the first injection, mice are boosted by injection with a second dose of the antigen mixed with incomplete Freund's adjuvant (IFA; lacks the killed mycobacterium of CFA).

A few weeks after the second injection, mice are tail bled and the sera titrated by immunoprecipitation against radiolabeled antigen. Preferably, the process of boosting and titrating is repeated until a suitable titer is achieved. The spleen of the mouse with the highest titer is removed and the spleen lymphocytes are obtained by

homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately 5×10^7 to 2×10^8 lymphocytes.

5 Mutant lymphocyte cells known as myeloma cells are obtained from laboratory animals in which such cells have been induced to grow by a variety of well-known methods. Myeloma cells lack the salvage pathway of nucleotide biosynthesis. Because myeloma cells are tumor cells, they can be propagated indefinitely in tissue culture, and are thus denominated immortal. Numerous cultured cell lines of myeloma cells from mice and rats, such as murine NS-1 myeloma cells, have been established.

10 Myeloma cells are combined under conditions appropriate to foster fusion with the normal antibody-producing cells from the spleen of the mouse or rat injected with the antigen/polypeptide of the present invention. Fusion conditions include, for example, the presence of polyethylene glycol. The resulting fused cells are hybridoma cells. Like myeloma cells, hybridoma cells grow indefinitely in culture.

15 Hybridoma cells are separated from unfused myeloma cells by culturing in a selection medium such as HAT media (hypoxanthine, aminopterin, thymidine). Unfused myeloma cells lack the enzymes necessary to synthesize nucleotides from the salvage pathway because they are killed in the presence of aminopterin, methotrexate, or azaserine. Unfused lymphocytes also do not continue to grow in tissue culture. Thus, only cells that have successfully fused (hybridoma cells) can grow in the selection media.

25 Each of the surviving hybridoma cells produces a single antibody. These cells are then screened for the production of the specific antibody immunoreactive with an antigen/polypeptide of the present invention. Single cell hybridomas are isolated by limiting dilutions of the hybridomas. The hybridomas are serially diluted many times and, after the dilutions are allowed to grow, the supernatant is tested for the presence of the monoclonal antibody. The clones producing that antibody are then cultured in large amounts to produce an antibody of the present invention in convenient quantity.

30 By use of a monoclonal antibody of the present invention, specific polypeptides and polynucleotide of the invention are identified as antigens. Once identified, those polypeptides and polynucleotide are isolated and purified by techniques such as antibody-affinity chromatography. In antibody-affinity

chromatography, a monoclonal antibody is bound to a solid substrate and exposed to a solution containing the desired antigen. The antigen is removed from the solution through an immunospecific reaction with the bound antibody. The polypeptide or polynucleotide is then easily removed from the substrate and purified.

5 Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. 5,223,409; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809, which are incorporated herein in their entirety by reference.

10 Additionally, recombinant anti-*Alloiococcus otitidis* antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human fragments, which are made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies are produced by recombinant DNA techniques known in the art, for
15 example using methods described in PCT/US86/02269; EP 184,187; EP 171,496; EP 173,494; WO 86/01533; U.S. 4,816,567; and EP 125,023.

 An anti-*Alloiococcus otitidis* antibody (*e.g.*, monoclonal antibody) is used to isolate *Alloiococcus otitidis* polypeptides by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-*Alloiococcus otitidis* antibody
20 facilitates the purification of a natural *Alloiococcus otitidis* polypeptide from cells and recombinantly produced *Alloiococcus otitidis* polypeptides expressed in host cells. Moreover, an anti-*Alloiococcus otitidis* antibody is used to detect *Alloiococcus otitidis* polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance of the *Alloiococcus otitidis* polypeptide. The detection of circulating
25 fragments of an *Alloiococcus otitidis* polypeptide is used to identify *Alloiococcus otitidis* polypeptide turnover in a subject. Anti-*Alloiococcus otitidis* antibodies are used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection is facilitated by coupling (*i.e.*, physically linking) the antibody to a
30 detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, P-galactosidase, or

acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and acquirin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{15}S or ^3H .

G. PHARMACEUTICAL COMPOSITIONS

10 In certain embodiments, the present invention provides pharmaceutical compositions comprising compounds that inhibit the activities of *Alloiococcus otitidis* polypeptides, and physiologically acceptable carriers. Compounds that inhibit the activities of *Alloiococcus otitidis* polypeptides, which are essential for
15 the proliferation of the bacteria, are identified using one or more assay systems set forth in Examples 5-38. More preferably, the pharmaceutical compositions comprise one or more compounds that inhibit the activities of *Alloiococcus otitidis* polypeptides comprising the amino acid residue sequence of one or more of the even numbered sequences set forth in SEQ ID NO: 2 through SEQ ID NO: 106. In other
20 embodiments, the pharmaceutical compositions of the invention comprise antisense polynucleotides of polynucleotides selected from one of the odd numbered sequences set forth in Seq. ID NO. 1 to Seq. ID No. 105, and physiologically acceptable carriers.

Various tests are to be used to assess the *in vitro* and *in vivo* efficacy of
25 antimicrobial and pharmaceutical compounds that inhibit the activities of *Alloiococcus otitidis* polypeptides, and these are set forth in detail in Examples 5 through 38. For example, an *in vitro* activity of the compounds may be assayed by incubating together a mixture of *Alloiococcus otitidis* or other heterologous bacterial cells such as *E. coli* cells expressing *Alloiococcus otitidis* polypeptides set forth in one of the
30 even numbered sequences from Seq. ID No. 2 to Seq. ID No. 106, and then measuring the activity of the polypeptide using one or more of the assay systems detailed in Example 5 through 38.

The *Alloiococcus otitidis* polynucleotides, polypeptides, compounds that modulate the activity of an *Alloiococcus otitidis* polypeptides, and anti-*Alloiococcus*

otitidis antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration to a host or subject, *e.g.*, a human. Such compositions typically comprise the nucleic acid molecule, protein, antimicrobial compound, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, (*e.g.*, intravenous, intradermal, subcutaneous, intraperitoneal), transmucosal (*e.g.*, oral, rectal, intranasal, vaginal, respiratory), and transdermal (topical). Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of

can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems.

Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods

30 H. DIAGNOSTIC ASSAYS

The invention also provides methods for detecting the presence of an *Alloiococcus otitidis* polypeptide or *Alloiococcus otitidis* polynucleotide, or fragment thereof, in a biological sample. The method involves contacting the biological sample

with a compound or an agent capable of detecting an *Alloiococcus otitidis* polypeptide or mRNA such that the presence of the *Alloiococcus otitidis* polypeptide/encoding nucleic acid molecule is detected in the biological sample. A preferred agent for detecting *Alloiococcus otitidis* mRNA or DNA is a labeled or labelable oligonucleotide probe capable of hybridizing to *Alloiococcus otitidis* mRNA or DNA. The nucleic acid probe can be, for example, a full-length *Alloiococcus otitidis* polynucleotide of one of the odd numbered sequences set forth in SEQ ID NO: 1 through SEQ ID NO: 105, a complement thereof, or a fragment thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to *Alloiococcus otitidis* mRNA or DNA. Alternatively, the sample can be contacted with an oligonucleotide primer of an *Alloiococcus otitidis* polynucleotide of SEQ ID NO: 1 through SEQ ID :105, a complement thereof, or a fragment thereof, in the presence of nucleotides and a polymerase, under conditions permitting primer extension.

A preferred agent for detecting *Alloiococcus otitidis* polypeptide is a labeled or labelable antibody capable of binding to an *Alloiococcus otitidis* polypeptide. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled or labelable," with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect *Alloiococcus otitidis* mRNA, DNA or protein in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of *Alloiococcus otitidis* mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of *Alloiococcus otitidis* polypeptide include enzyme linked immunosorbent assays (ELISAs), Western-blots, immunoprecipitations and immunofluorescence. Alternatively, *Alloiococcus otitidis*

polypeptides can be detected *in vivo* in a subject by introducing into the subject a labeled anti-*Alloicoccus otitidis* antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

5 The polynucleotides according to the invention may also be used in analytical DNA chips, which allow sequencing, the study of mutations and of the expression of genes, and which are currently of interest given their very small size and their high capacity in terms of number of analyses.

10 The principle of the operation of these chips is based on molecular probes, most often oligonucleotides, which are attached onto a miniaturized surface, generally of the order of a few square centimeters. During an analysis, a sample containing fragments of a target nucleic acid to be analyzed, for example DNA or RNA labeled, for example, after amplification, is deposited onto the DNA chip in which the support has been coated beforehand with probes. Bringing the labeled
15 target sequences into contact with the probes leads to the formation, through hybridization, of a duplex according to the rule of pairing defined by J.D. Watson and F. Crick. After a washing step, analysis of the surface of the chip allows the effective hybridizations to be located by means of the signals emitted by the labels tagging the target. A hybridization fingerprint results from this analysis which, by appropriate
20 computer processing, will make it possible to determine information such as the presence of specific fragments in the sample, the determination of sequences and the presence of mutations.

25 The chip consists of a multitude of molecular probes, precisely organized or arrayed on a solid support whose surface is miniaturized. It is at the center of a system where other elements (imaging system, microcomputer) allow the acquisition and interpretation of a hybridization fingerprint.

30 The hybridization supports are provided in the form of flat or porous surfaces (pierced with wells) composed of various materials. The choice of a support is determined by its physicochemical properties, or more precisely, by the relationship between the latter and the conditions under which the support will be placed during the synthesis or the attachment of the probes or during the use of the chip. It is therefore necessary, before considering the use of a particular support, to consider characteristics such as its stability to pH, its physical strength, its reactivity and its

chemical stability as well as its capacity to nonspecifically bind nucleic acids.

Materials such as glass, silicon and polymers are commonly used. Their surface is, in a first step, called "functionalization", made reactive towards the groups which it is desired to attach thereon. After the functionalization, so-called spacer molecules are grafted onto the activated surface. Used as intermediates between the surface and the probe, these molecules of variable size render unimportant the surface properties of the supports, which often prove to be problematic for the synthesis or the attachment of the probes and for the hybridization.

Among the hybridization supports, there may be mentioned glass which is used, for example, in the method of *in situ* synthesis of oligonucleotides by photochemical addressing developed by the company Affymetrix (E.L. Sheldon, 1993), the glass surface being activated by silane. Genosensor Consortium (P. Mérel, 1994) also uses glass slides carrying wells 3 mm apart, this support being activated with epoxysilane.

The probes according to the invention may be synthesized directly *in situ* on the supports of the DNA chips. This *in situ* synthesis may be carried out by photochemical addressing (developed by the company Affymax (Amsterdam, Holland) and exploited industrially by its subsidiary Affymetrix (United States)) or based on the VLSIPS (very large scale immobilized polymer synthesis) technology (S.P.A. Fodor *et al.*, 1991) which is based on a method of photochemically directed combinatorial synthesis and the principle of which combines solid-phase chemistry, the use of photolabile protecting groups and photolithography.

The probes according to the invention may be attached to the DNA chips in various ways such as electrochemical addressing, automated addressing or the use of probe printers (T. Livache *et al.*, 1994; G. Yershov *et al.*, 1996; J. Derisi *et al.*, 1996, and S. Borman, 1996).

The revealing of the hybridization between the probes of the invention, deposited or synthesized *in situ* on the supports of the DNA chips, and the sample to be analyzed, may be determined, for example, by measurement of fluorescent signals, by radioactive counting or by electronic detection.

The use of fluorescent molecules such as fluorescein constitutes the most common method of labeling the samples. It allows direct or indirect revealing of the hybridization and allows the use of various fluorochromes.

Affymetrix currently provides an apparatus or a scanner designed to read its Gene Chip™ chips. It makes it possible to detect the hybridizations by scanning the surface of the chip in confocal microscopy (R.J. Lipshutz *et al.*, 1995).

The nucleotide sequences according to the invention are also used in DNA chips to carry out the analysis of the expression of the *Alloiococcus otitidis* genes. This analysis of the expression of *Alloiococcus otitidis* genes is based on the use of chips where probes of the invention, chosen for their specificity to characterize a given gene, are present (D.J. Lockhart *et al.*, 1996; D.D. Shoemaker *et al.*, 1996). For the methods of analysis of gene expression using the DNA chips, reference may, for example, be made to the methods described by D.J. Lockhart *et al.* (1996) and Sosnowsky *et al.* (1997) for the synthesis of probes *in situ* or for the addressing and the attachment of previously synthesized probes. The target sequences to be analyzed are labeled and in general fragmented into sequences of about 50 to 100 nucleotides before being hybridized onto the chip. After washing as described, for example, by D.J. Lockhart *et al.* (1996) and application of different electric fields (Sosnowsky *et al.*, 1997), the labeled compounds are detected and quantified, the hybridizations being carried out at least in duplicate. Comparative analyses of the signal intensities obtained with respect to the same probe for different samples and/or for different probes with the same sample, determine the differential expression of RNA or of DNA derived from the sample.

The nucleotide sequences according to the invention are, in addition, used in DNA chips where other nucleotide probes specific for other microorganisms are also present, and allow the carrying out of a serial test allowing rapid identification of the presence of a microorganism in a sample:

Accordingly, the subject of the invention is also the nucleotide sequences according to the invention, characterized in that they are immobilized on a support of a DNA chip.

The DNA chips, characterized in that they contain at least one nucleotide sequence according to the invention, immobilized on the support of the said chip, also form part of the invention.

The chips preferably contain several probes or nucleotide sequences of the invention of different length and/or corresponding to different genes so as to identify, with greater certainty, the specificity of the target sequences or the desired mutation

in the sample to be analyzed.

Accordingly, the analyses carried out by means of primers and/or probes according to the invention, immobilized on supports such as DNA chips, make it possible, for example, to identify, in samples, mutations linked to variations such as intraspecies variations. These variations may be correlated or associated with pathologies specific to the variant identified and make it possible to select the appropriate treatment.

The invention thus comprises a DNA chip according to the invention, characterized in that it contains, in addition, at least one nucleotide sequence of a microorganism different from *Alloiococcus otitidis*, immobilized on the support of the said chip; preferably, the different microorganism is chosen from an associated microorganism, a bacterium of the *Streptococcus* family, and a variant of the species *Alloiococcus otitidis*.

The principle of the DNA chip as explained above, is also used to produce protein "chips" on which the support has been coated with a polypeptide or an antibody according to the invention, or arrays thereof, in place of the DNA. These protein "chips" make it possible, for example, to analyze the biomolecular interactions (BIA) induced by the affinity capture of target analytes onto a support coated, for example, with proteins, by surface plasma resonance (SPR). Reference may be made, for example, to the techniques for coupling proteins onto a solid support which are described in EP 524 800 or to the methods describing the use of biosensor-type protein chips such as the BIAcore-type technique (Pharmacia) (Arlinghaus *et al.*, 1997, Krone *et al.*, 1997, Chatelier *et al.*, 1995). These polypeptides or antibodies according to the invention, capable of specifically binding antibodies or polypeptides derived from the sample to be analyzed, are thus used in protein chips for the detection and/or the identification of proteins in samples. The said protein chips may in particular be used for infectious diagnosis and preferably contain, per chip, several polypeptides and/or antibodies of the invention of different specificity, and/or polypeptides and/or antibodies capable of recognizing microorganisms different from *Alloiococcus otitidis*.

Accordingly, the subject of the present invention is also the polypeptides and the antibodies according to the invention, characterized in that they are immobilized on a support, in particular, on a protein chip.

The protein chips, characterized in that they contain at least one polypeptide or one antibody according to the invention immobilized on the support of the said chip, also form part of the invention.

The invention comprises, in addition, a protein chip according to the invention,
5 characterized in that it contains, in addition, at least one polypeptide of a microorganism different from *Alloicoccus otitidis* or at least one antibody directed against a compound of a microorganism different from *Alloicoccus otitidis*, immobilized on the support of the chip.

The invention also relates to a kit or set for the detection and/or the
10 identification of bacteria belonging to the species *Alloicoccus otitidis* or to an associated microorganism, or for the detection and/or the identification of a microorganism characterized in that it comprises a protein chip according to the invention.

The present invention also provides a method for the detection and/or the
15 identification of bacteria belonging to the species *Alloicoccus otitidis* or to an associated microorganism in a biological sample, characterized in that it uses a nucleotide sequence according to the invention.

The invention also encompasses kits for detecting the presence of an *Alloicoccus otitidis* polypeptide in a biological sample. For example, the kit
20 comprises reagents such as a labeled or labelable compound or agent capable of detecting *Alloicoccus otitidis* polypeptide or mRNA in a biological sample; means for determining the amount of *Alloicoccus otitidis* polypeptide in the sample; and means for comparing the amount of *Alloicoccus otitidis* polypeptide in the sample with a standard. The compound or agent are packaged in a suitable container. The kit
25 further comprises instructions for using the kit to detect *Alloicoccus otitidis* mRNA or protein.

In certain embodiments, detection involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g. U.S. 4,683,195 and U.S. 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction
30 (LCR). This method includes the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to an *Alloicoccus otitidis* polynucleotide under conditions such that

hybridization and amplification of the *Alloiococcus otitidis*-polynucleotide (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample.

5

I. TRANSGENIC ANIMALS

It is contemplated that in some instances the genome of a transgenic animal of the present invention will have been altered through the stable introduction of one or more of the *Alloiococcus otitidis* polynucleotide compositions described herein, either native, synthetically modified or mutated. As described herein, a "transgenic animal" refers to any animal, preferably a non-human mammal (e.g. mouse, rat, rabbit, squirrel, hamster, rabbits, guinea pigs, pigs, micro-pigs, baboons, squirrel monkeys and chimpanzees, etc), bird or an amphibian, in which one or more cells contain a heterologous nucleic acid sequence introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly, by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical crossbreeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA.

The host cells of the invention are also used to produce non-human transgenic animals. The non-human transgenic animals are used in screening assays designed to identify infections or compounds, e.g., drugs, pharmaceuticals, etc., which are capable of ameliorating *Alloiococcus otitidis* symptoms or infections. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which an *Alloiococcus otitidis* polypeptide-coding sequence has been introduced. Such host cells are then used to create non-human transgenic animals in which exogenous *Alloiococcus otitidis* gene sequences have been introduced into their genome or homologous recombinant animals in which endogenous *Alloiococcus otitidis* gene sequences have been altered. Such animals are useful for studying the effects of an *Alloiococcus otitidis* polypeptide and for

identifying and/or evaluating modulators of *Alloiococcus otitidis* polypeptide infectivity.

A transgenic animal of the invention is created by introducing an *Alloiococcus otitidis* polypeptide-encoding nucleic acid sequence into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human *Alloiococcus otitidis* cDNA sequence of one or more of SEQ ID NO:1 through SEQ ID NO: 4035 can be introduced as a transgene into the genome of a non-human animal.

Moreover, a non-*Alloiococcus otitidis* homologue of the *Alloiococcus otitidis* gene can be isolated based on hybridization to the *Alloiococcus otitidis* cDNA (described above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the *Alloiococcus otitidis* transgene to direct expression of an *Alloiococcus otitidis* polypeptide to particular cells. Methods for generating transgenic animals *via* embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. 4,736,866 and 4,870,009, U.S. 4,873,191 and in Hogan, 1986. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the *Alloiococcus otitidis* transgene in its genome and/or expression of *Alloiococcus otitidis* mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding an *Alloiococcus otitidis* polypeptide can further be bred to other transgenic animals carrying other transgenes.

In another embodiment, transgenic non-human animals can be produced which contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P λ . For a description of the cre/loxP recombinase system, *see, e.g.*, Lakso *et al.*, 1992. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gonnan *et al.*, 1991). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required.

Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

5 Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.*, 1997, and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through
10 the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is
15 isolated.

All patents and publications cited herein are hereby incorporated by reference.

The following examples are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described
20 in detail. The following examples are presented for illustrative purposes, and should not be construed in any way limiting the scope of this invention.

EXAMPLE 1

CONFIRMATION OF THE IDENTITY OF THE *ALLOIOCOCCUS OTITIDIS* 1104-92 ISOLATE

25

The *Alloiococcus otitidis* isolate 1104-92 was obtained from Dr. Richard Facklam of the Centers for Disease Control in Atlanta. It was isolated from the middle ear fluid of a child in the Atlanta, Georgia area. It was confirmed to be *A. otitidis* by comparing it to the type strain, ATCC51267, obtained from the American Type
30 Culture Collection [Aguirre, 1992 #1]. Both the 1104-92 and type strain are characterized as Gram positive cocci. Both grow on Columbia agar supplemented with 5% yeast extract, 0.5% polysorbate 80 (Tween 80), and 0.7% phosphatidyl choline when incubated at 37°C. On this medium, both strains form slow growing

small white colonies that require nearly two days to be easily observed with the naked eye. Both are sensitive to lysis by hen egg white lysozyme and *Streptococcus globisporus* mutanolysin. Both grow in the presence of 2% sodium azide. Both are killed by incubation at 55°C for 30 minutes. Finally, to further confirm that the 1104-92 was a strain of *A. otitidis*, it was subject to polymerase chain reaction (PCR) identification based on its 16s rRNA gene. This was done using two of the primers specified by Aguirre and Collins [Aguirre, 1992 #2]. The antisense primer used was 5'-ATCTTCCTGCTTGCAGGAAGAGG-3' and the sense primer was 3'-CGCTTCATCTCTGAAGCTAGC-5'. Thus by multiple criteria, the 1104-92 strain was confirmed to be an isolate of *A. otitidis*.

EXAMPLE 2

STORAGE, GROWTH, AND HARVEST OF ALLOIOCOCCUS OTTIDIS 1104-92 FOR ISOLATION OF DNA

The *A. otitidis* isolate 1104-92 was stored at -70°C in Todd-Hewlett broth containing 40% glycerol. A small portion of the frozen stock was streaked onto the agar medium described in Example 1 and incubated at 37°C for two days. The growth from the plate was swabbed into a 17 × 100 cm tube containing 6 ml of a serum-free broth medium. This broth medium was prepared with 30 g Todd-Hewlett medium, 5 g yeast extract, 10 ml polysorbate 80 (Tween 80), and 1 liter distilled water. This medium was sterilized by autoclaving for 35 minutes. The bacteria were incubated aerobically without shaking in an aerobic incubator at 37°C for two days. The tube containing the growing bacteria was then shaken to resuspend the bacteria and added to a liter of the same medium in a Fernbach flask. This flask, in turn, was incubated aerobically for three days without shaking. The bacteria were harvested by first swirling the flask to suspend the bacteria and then low speed centrifugation at about 5,000 × g for 30 minutes. The pellet of bacteria was washed by resuspending it in 10 to 15 mL of phosphate buffered saline (PBS), and centrifuging the suspension at about 8,000 × g for 20 minutes. The pellet of bacteria was retained and stored frozen at -20°C. The yield of wet bacterial pellet was typically about 1 g per liter of broth.

EXAMPLE 3

PREPARATION OF *ALLOIOCOCCUS OTITIDIS* GENOMIC DNA

To prepare genomic DNA, 0.95 g frozen pellet of bacteria was defrosted and
5 suspended in 10 mL of PBS containing 1 mM MgCl₂. The bacteria were killed by
incubating the suspension at 55°C for 20 minutes. The suspension was allowed to
cool before adding 25 µl of a 10 mg/mL stock of hen egg white lysozyme and 50 µl of
a 25,000 unit/mL stock of *Streptococcus globisporus* mutanolysin to the suspension.
It was then incubated for one hour at 37°C. Then 50 µl of a 10 mg/mL stock of
10 RNase was added and the suspension incubated an additional hour at 37°C. After
these incubations, sodium dodecylsulfate (SDS) was added to a final concentration
of 0.3% (0.3 mL of a 10% stock). This was followed by the addition of 0.3 mL of a 1
mg/mL stock of proteinase K. The suspension was then incubated for two hours at
37°C. After this time, an equal volume of water saturated phenol/chloroform/isopropyl
15 (25:24:1) was added to the digested suspension and gently mixed. The upper
aqueous layer was retained after a low speed centrifugation and 2.5 volumes of
ethanol were added and the tube gently inverted to mix. The DNA was then spooled
out on a glass rod and allowed to air dry.

The DNA at this stage still contained obvious impurities and needed further
20 purification. The DNA dried on the glass rod was soaked in 70% ethanol to remove
excess phenol and air-dried once again. It was then suspended in 2 ml of Tris-EDTA
buffer to which 2 µl of RNase cocktail was added and incubated at room temperature
for 75 minutes. Then 100 µl of protease, 100 µl SDS and 40 µl of 100 mM CaCl₂
were added and the suspension incubated for 3.5 hours. An equal volume of
25 chloroform was added, gently mixed, then centrifuged at a low speed. The aqueous
layer was collected and re-extracted with the phenol, chloroform, isopropyl alcohol
reagent. In turn, the aqueous layer was extracted with chloroform. At this point, 3 M
sodium acetate was added to the aqueous phase collected from the last extraction
and then 3.75 ml of ethanol was added and gently mixed. The DNA was spooled out,
30 soaked in 70% ethanol and allowed to air-dry. The DNA was finally suspended in 2
ml of Tris-EDTA buffer. Based on absorption at 260 nm, the final yield of DNA was
482 µg of DNA. The DNA was confirmed to be that of *A. otitidis* by the PCR method
described in example 1. This DNA was submitted for sequencing.

EXAMPLE 4

CLONING AND SEQUENCING ALLOIOCOCCUS OTITIDIS GENOME

5 This invention provides nucleotide sequences of the genome of *Alloiococcus otitidis* which thus comprises a DNA sequence library of *Alloiococcus otitidis* genomic DNA. The detailed description that follows provides nucleotide sequences of *Alloiococcus otitidis*, and also describes how the sequences were obtained and how ORFs (Open Reading Frames) and protein-coding sequences can be identified.

10 To construct a library, genomic DNA was hydrodynamically sheared in an HPLC and then separated on a standard 1% agarose gel. A fraction corresponding to 3000-3500 bp in length was excised from the gel and purified by the GeneClean procedure (BIO101, Inc.).

The purified DNA fragments were then blunt-ended using T4 DNA
15 polymerase. The blunt-ended DNA was then ligated to unique BstX1-linker adapters. These linkers are complimentary to the pGTC vector, while the overhang is not self-complimentary. Therefore, the linkers will not concatemerize nor will the cut-vector religate itself easily. The linker-adapted inserts were separated from the unincorporated linkers on a 1% agarose gel and again purified using GeneClean.
20 The linker-adapted inserts were then ligated to BstX1-cut vector to construct "shotgun" subclone libraries.

Only major modifications to the protocols are highlighted. Briefly, the library was transformed into DH10B competent cells (Gibco/BRL, DH5a transformation protocol). Transformed cells were detected by plating onto antibiotic plates
25 containing ampicillin. The plates were incubated overnight at 37° C. Transformant clones were then selected for sequencing. The cultures were grown overnight at 37°C. DNA was purified using a silica bead DNA preparation (Egelstein, 1996) method. In this manner, 25 mg of DNA was obtained per clone.

These purified DNA samples were then sequenced using ABI dye-terminator
30 chemistry. All subsequent steps were based on sequencing by automated DNA sequencing methods. The ABI dye terminator sequence reads were run on MegaBace™ 10000 (Amersham) machines and the data transferred to UNIX based computers. Base calls and quality scores were determined using the PHRED

software program (Ewing et al., 1998, Genome Res. 8: 175-185; Ewing and Green, 1998, Genome Res. 8:685-734). Reads were assembled using PHRAP (P. Green, Abstracts of DOE Human Genome Program Contractor-Grantee Workshop V, Jan. 1996, p 157) with default program parameters and quality scores.

5 To identify *Alloiococcus otitidis* genome encoded polypeptides, the complete genomic sequence of *Alloiococcus otitidis* was analyzed essentially as follows: First, all possible stop-to-stop open reading frames (ORFs) ≥ 222 nucleotides in all three reading frames were translated into amino acid sequences.

10 Second, the identified ORFs were analyzed for homology to known protein sequences. Third, the coding potential of non-homologous sequences were evaluated with the GENEMARK™ software program (Borodovsky and McIninch, 1993, Comp. Chem. 17:123). The results of these analysis are set forth in tables 2-16.

15

EXAMPLE 5

IDENTIFICATION OF SPECIFIC GENES IN ALLOIOCOCCUS OTITIDIS

Alloiococcus otitidis homologs of the genes listed in Table 4 were identified as follows:

20 Protein sequences of interest ("query sequences", Table 4) were extracted from Genbank from one or more species; query species included but were not limited to *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Lactococcus lactis*, *Escherichia coli*, and *Bacillus subtilis*. These queries were compared to the *Alloiococcus otitidis* sequence by several methods in order to
25 determine which *Alloiococcus* sequence was the ortholog for the query gene.

First, the query sequences were compared to the translated *Alloiococcus otitidis* ORF set using BLASTP. The ORF set was generated as described in Vaccines patent, except that for each ORF that had multiple potential start codons, only the longest ORF was used. The top 10 *Alloiococcus otitidis* hits for each query
30 were saved, without regard to score.

These *Alloiococcus otitidis* hits were then compared to NR, the nonredundant Genpept database, using BLASTP. An *Alloiococcus otitidis* ORF was considered the ortholog of a query sequence if the genes were reciprocal best hits in *Alloiococcus*

otitidis and the query genome. This analysis is also summarized in Table 4 (excel file AOT_PATENT_FILE.xls, Sheet TopHitsAndClustalKey). Specific numerical cutoffs were not used; however all top hits had Expect values of less than 3×10^{-28} .

Several query sequences had more than one high-scoring hit in *Alloicoccus*
5 *otitidis*. In most cases, however, only the first, best hit to the original query sequence had that query sequence as its reciprocal best hit. For example, the *Streptococcus pyogenes* query sequence GyrA (alpha subunit of DNA gyrase) has two high-scoring hits in *Alloicoccus otitidis*. These were distinguished by the reciprocal blast analysis; the first, ORF_505 (60% identity, Expect = 0) is the GyrA homolog and the
10 second, ORF_1907 (38% identity, Expect = 1×10^{-154}) is the homolog of the query sequence GrlA or ParC (topoisomerase IV, A subunit). Other examples of closely related proteins include the B subunits of DNA gyrase (GyrB) and Topoisomerase IV (GrlB or ParE); and YphC and Era, both of which are putative GTP binding proteins of unknown function. These *Alloicoccus otitidis* ORFS were assigned based on
15 their top hit in Genpept.

In two cases the multiple high-scoring hits in *Alloicoccus otitidis* were the result of gene duplication. In the case of MurA (UDP-N-acetylglucosamine enolpyruvyl transferase) two separate *Alloicoccus otitidis* ORFS were determined to be the desired orthologs, because both had MurA (or MurZ, alternate notation) as
20 their best hit in Genpept. Likewise, there are two FolC (folylpolyglutamate synthase) homologs in *Alloicoccus otitidis*. It is known that other bacteria, particularly Gram-positive bacteria, may carry two homologs of each of these genes.

As a further step in verification of gene assignments, the *Alloicoccus otitidis* ORFS identified as orthologs of the query genes by the analysis above were then
25 compared to an internal copy of the COGS database (Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV, 2001, Nucleic Acids Res 2001 Jan 1;29(1):22-8. The COG database: new developments in phylogenetic classification of proteins from complete genomes) using BLASTP. The COGS database is a curated set of proteins
30 from a set of finished bacterial genomes, which have been grouped into specific protein families on the basis of protein similarity. In all cases, the *Alloicoccus otitidis* ORF was most closely related to the COGS family of the initial query protein, if that protein had been assigned to a COGS family. Examples of proteins for which

there is no COGS family defined (in our local version of the database) include SrtA (sortase) and MvaK1 (phosphomevalonate kinase).

As a final confirmation, all query proteins were compared to the complete *Alloiococcus otitidis* nucleotide sequence using TBLASTN, in order to determine if
5 there were additional and/or better hits that had not been predicted as ORFS. In all cases, the same sequence was identified as the best hit by TBLASTN and by BLASTP.

For one query sequence, sortase, the *Alloiococcus otitidis* ORF that was the top hit (Expect = 0.42) by the initial BLASTP or TBLASTN using the *Staphylococcus aureus* sortase sequence as query was found by additional analysis (reciprocal blast)
10 to be a putative ABC-transport protein. The true sortase homolog in *Alloiococcus otitidis* was identified by construction of a Hidden Markov Model based on a multiple alignment of 72 known and putative sortase proteins that had been identified previously using similar computational methods. The model was constructed using
15 "hmmbuild" and the *Alloiococcus otitidis* ORF set was searched using "hmmsearch", both of the hmmer package (S.R. Eddy. Profile hidden Markov models. *Bioinformatics* 14:755-763, 1998). The assignment of ORF_876 as sortase was then confirmed by reciprocal blast as described above and in Table 2. ORF_876 was also found to be the top hit in *Alloiococcus otitidis* when the *Bacillus subtilis* putative
20 sortase (YhcS) was used as the query sequence in a BLASTP search. The *Bacillus halodurans* BH3596 *Bacillus subtilis* YhcS and proteins that are the top hits for RF_876 have recently been placed into a COGS group of sortases, further confirming the identity of ORF_876 as the *Alloiococcus otitidis* sortase.

TABLE 4

ORF NO.	DNA SEQ ID NO.r	ORF Start	Protein Start	ORF Stop	Protein SEQ ID No.	Gene
46b	Seq. ID No. 1	26225	26153	25800	Seq. ID No. 2	ACPS
48c	Seq. ID No. 3	29105	29090	27696	Seq. ID No. 4	murF
57b	Seq. ID No. 5	33738	33732	32455	Seq. ID No. 6	murA-2
65c	Seq. ID No. 7	37245	37242	36634	Seq. ID No. 8	rpoE
172	Seq. ID No. 9	88726	88726	87785	Seq. ID No. 10	rpoA
228d	Seq. ID No. 11	111563	111542	107883	Seq. ID No. 12	rpoC
236c	Seq. ID No. 13	115224	115221	111643	Seq. ID No. 14	rpoB
495c	Seq. ID No. 15	247355	247331	245949	Seq. ID No. 16	dnaB/C
505b	Seq. ID No. 17	254277	254268	251794	Seq. ID No. 18	gyrA
509	Seq. ID No. 19	256252	256246	254297	Seq. ID No. 20	gyrB
515c	Seq. ID No. 21	259131	259116	257914	Seq. ID No. 22	dnaN
528b	Seq. ID No. 23	263837	263861	265153	Seq. ID No. 24	folC-2
851b	Seq. ID No. 25	440982	441072	442634	Seq. ID No. 26	murE
876	Seq. ID No. 27	453874	453898	454509	Seq. ID No. 28	srtA
956	Seq. ID No. 29	500019	500019	501308	Seq. ID No. 30	folC-1
959	Seq. ID No. 31	501978	501993	502364	Seq. ID No. 32	folB
961c	Seq. ID No. 33	502392	502413	502943	Seq. ID No. 34	folK
1183b	Seq. ID No. 35	626391	626430	627632	Seq. ID No. 36	mvaS
1184	Seq. ID No. 37	629315	629285	627993	Seq. ID No. 38	mvaA
1263	Seq. ID No. 39	675596	675608	676525	Seq. ID No. 40	murB
1273	Seq. ID No. 41	685392	685377	684289	Seq. ID No. 42	mvaK2
1275b	Seq. ID No. 43	686415	686403	685393	Seq. ID No. 44	mvaD
1277	Seq. ID No. 45	687376	687349	686396	Seq. ID No. 46	mvaK1
1279	Seq. ID No. 47	687461	687506	688435	Seq. ID No. 48	coaA
1284	Seq. ID No. 49	691675	691681	692520	Seq. ID No. 50	nadE
1511	Seq. ID No. 51	815078	815084	815920	Seq. ID No. 52	murI
1811b	Seq. ID No. 53	985498	985504	986454	Seq. ID No. 54	folP
1863b	Seq. ID No. 55	1019023	1019050	1019583	Seq. ID No. 56	folA
1902	Seq. ID No. 57	1040639	1040645	1042606	Seq. ID No. 58	GrIB
1907	Seq. ID No. 59	1042729	1042732	1045191	Seq. ID No. 60	grIA

Table 4
(Cont'd.)

ORF NO.	DNA SEQ ID NO.r	ORF Start	Protein Start	ORF Stop	Protein SEQ ID No.	Gene
1990c	Seq. ID No. 61	1098801	1098798	1097689	Seq. ID No. 62	rpoD
1992b	Seq. ID No. 63	1100670	1100670	1098817	Seq. ID No. 64	dnaG
2003	Seq. ID No. 65	1109198	1109144	1108212	Seq. ID No. 66	era
2016h	Seq. ID No. 67	1115435	1115390	1113879	Seq. ID No. 68	norA
2133	Seq. ID No. 69	1179995	1179938	1175604	Seq. ID No. 70	polC
2181b	Seq. ID No. 71	1203606	1203588	1202281	Seq. ID No. 72	obg
2204	Seq. ID No. 73	1216828	1216804	1215491	Seq. ID No. 74	yphC
2240c	Seq. ID No. 75	1236616	1236607	1233293	Seq. ID No. 76	dnaE
2284	Seq. ID No. 77	1261069	1261063	1259858	Seq. ID No. 78	coaBC
2328	Seq. ID No. 79	1286689	1286668	1285637	Seq. ID No. 80	holA
2333	Seq. ID No. 81	1290847	1290847	1290371	Seq. ID No. 82	coaD
2485	Seq. ID No. 83	1374427	1374400	1373168	Seq. ID No. 84	ftsZ
2489	Seq. ID No. 85	1375804	1375792	1374428	Seq. ID No. 86	ftsA
2492b	Seq. ID No. 87	1378075	1378060	1376897	Seq. ID No. 88	murG
2494	Seq. ID No. 89	1379477	1379453	1378050	Seq. ID No. 90	murD
2514	Seq. ID No. 91	1390141	1390135	1389491	Seq. ID No. 92	nadD
2596	Seq. ID No. 93	1437374	1437374	1436709	Seq. ID No. 94	coaE
2602	Seq. ID No. 95	1442399	1442396	1441065	Seq. ID No. 96	murC
2645	Seq. ID No. 97	1467800	1467782	1466751	Seq. ID No. 9	fmhB
2875	Seq. ID No. 99	1605944	1605923	1603701	Seq. ID No. 100	pcrA
2918	Seq. ID No. 101	1631092	1631089	1629779	Seq. ID No. 102	murA-1
3001	Seq. ID No. 103	1680254	1680221	1679229	Seq. ID No. 104	holB
3012	Seq. ID No. 105	1684114	1684102	1682330	Seq. ID No. 106	dnaX

5

EXAMPLE 6

IDENTIFICATION OF THE GENE ENCODING COENZYME A (COA) IN ALLOIOCOCCUS OTITIDIS

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Pantothenate kinase (Pank, CoaA) encoded by the *coaA* gene catalyzes the initial step in Coenzyme A (CoA) biosynthesis. CoA is an essential co-factor in a number of metabolic pathways in bacteria and mammals. Short-chain thioesters such as acetyl-CoA and succinyl-CoA are essential intermediates in carbon metabolism. CoA-thioesters of long chain fatty acids feed into β -oxidation and are also the source of fatty acids for phospholipids. In addition, CoA and its thioesters play important roles in the regulation of several enzymes in intermediary metabolism, including pyruvate dehydrogenase and phosphoenolpyruvate carboxylase. Finally,

15

synthesis of holo acyl carrier protein (ACP) is dependent on CoA for the 4'-phosphopantetheine moiety linked to ACP. ACP is essential for fatty acid biosynthesis. The two major acyl-carrier groups in cells: CoA and ACP, are derived from pantothenate. Pantothenate can be obtained exogenously through uptake via a permease, the product of the *panF* gene. Alternately, pantothenate is the product of condensation of pantoate and β -alanine via pantothenate synthetase, the product of the *panC* gene. The initial step in CoA biosynthesis is the phosphorylation of pantothenate by pantothenate kinase (PanK, CoaA).

The *coaA* gene was originally identified by Dunn and Snell in *S. typhimurium* as a temperature sensitive allele. Similarly, a temperature sensitive allele of *coaA* was reported for *E. coli* in 1987. CoaA was found to be essential in *E. coli* in a recent genetic footprinting analysis. In the temperature sensitive strains, accumulation of phosphorylated CoA intermediates rapidly ceased following shift to the non-permissive temperature. CoaA was shown to be a homo-dimer of 35 kDa subunits that bound ATP cooperatively. ATP is bound first in a sequential mechanism of action; CoA has been shown to be a potent inhibitor of the reaction and competitively competes for binding with ATP. Therefore CoaA is under feedback regulation and is the major regulatory step in CoA biosynthesis.

Lysine 101 in bacterial pantothenate kinase (CoaA) was found to be essential for both ATP and CoA binding. This supports kinetic data that CoA is a competitive inhibitor of ATP binding to CoaA and that both substrates bind to the same site.

Homologues of *E. coli* CoaA have been identified in *B. subtilis*, *S. pyogenes*, *M. tuberculosis*, *H. influenzae* and *V. cholerae*. Homologues have not been identified in either the *S. cerevisiae* genome or in a mammalian expressed sequence tag database. Calder *et al.* identified a homologue, through functional complementation of an *E. coli coaA* ts mutant, in *A. nidulans*. Homologue of this gene identified in *Alloiococcus otitidis* as described in Example 5 (Seq. ID No 47. The protein encoded by the gene is set forth in Seq. ID No. 48.

The *A. nidulans* gene was then used to identify a yeast homologue. The bacterial and Aspergillus enzymes were found to be 16% identical and 32% similar. Although this level of similarity is quite weak the essential lysine residue involved in nucleotide binding appears to be conserved; however, the sequence surrounding the lysine residue were not conserved and further study will be required to validate this

finding. The most striking difference between the eukaryotic and prokaryotic enzymes is found in the sensitivity of each to competitive inhibition by CoA and acetyl-CoA. The yeast enzyme was most sensitive to acetyl-CoA and less sensitive to CoA, whereas the converse was true for the bacterial enzyme. Later studies
5 demonstrated that mammalian pantothenate kinase is activated by CoA and inhibited by acetyl-CoA.

Nucleotide binding

Binding of ATP to CoaA is directly demonstrated by equilibrium dialysis
10 employing the non-hydrolyzable ATP analogue ATP γ S. The K_d measured for ATP binding is reported to be 2.1 μ M.

CoA binding

Binding of CoA to CoaA is directly demonstrated by equilibrium dialysis and
15 the K_d is reported to be 6.7 μ M.

Pantothenate kinase activity

Specific kinase activity of CoaA is demonstrated using D-[1- 14 C]pantothenate and capturing 4'-phospho[1- 14 C]pantothenate on DE81 filters. Using this assay the
20 following kinetic values were derived: specific activity – 470 \pm 200 nmol/min/mg; pantothenate K_m – 36 μ M; K_m ATP – 136 μ M.

Suitability of target for anti-infective development

Coenzyme A biosynthesis is essential for bacterial viability. CoaA catalyzes the
25 first step of biosynthesis of CoA and appears to be the point of regulation for the pathway. The essentiality of CoaA is demonstrated through the construction of temperature sensitive alleles in *coaA*. Although the yeast enzyme is found to functionally complement the bacterial temperature sensitive allele, sequence and kinetic differences suggest the possibility of identifying inhibitors of the bacterial
30 enzyme with high selectivity. As CoaA is essential and conserved in gram-negative and gram-positive pathogens, such inhibitors will have broad-spectrum utility.

Suitable assays for measuring CoaA function

CoaA is purified by standard methods using widely available molecular tags following expression at high level from *E. coli*. Pantothenate kinase activity is measured as follows: CoaA and D-[1-¹⁴C]pantothenate is incubated in a buffer
5 consisting of 100 mM Tris (pH 7.4), 2.5 mM MgCl₂, 2.5 mM ATP for 5-60 minutes at 37°C. Product, 4'-phospho[1-¹⁴C] pantothenate, is monitored through retention of labeled material on DE81 filters. This assay is amenable to high-throughput screening using high-density well-filter plates.

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EXAMPLE 7**IDENTIFICATION OF THE GENE ENCODING COABC (DFP) IN *ALLOIOCOCCUS OTTIDIS***

The *E. coli dfp* gene, which encodes the previously designated Dfp protein, was originally identified as encoding an enzyme required for CoA biosynthesis. The
15 gene, coding for the protein of interest, was renamed *coaBC* to reflect the enzyme function in CoA biosynthesis. CoA is an essential co-factor in a number of metabolic pathways in bacteria and mammals. Short-chain thioesters such as acetyl-CoA and succinyl-CoA are essential intermediates in carbon metabolism.

CoaBC carries out the second and third steps of coenzyme A
20 biosynthesis: the conjugation of 4'-phosphopantetheate with cysteine by the CoaB (PPCS : 4'-phosphopantetheenoyl cysteine synthase) activity followed by the conversion to 4'-phosphopantetheine by the CoaC (PPCDC: 4'-phosphopantenoylcysteine decarboxylase) activity. Homologue of this gene identified in *Alloioococcus otitidis* as described in Example 5 (Seq. ID No 77). The
25 protein encoded by the gene is set forth in Seq. ID No. 78.

Enzyme activity of CoaBC (Dfp):

Initially it was demonstrated that Dfp enzyme catalyzing oxidative
30 decarboxylation of (R)-4'-phospho-N-pantothenoylcysteine (PPC) to form 4'-phosphopantetheine (PP) – the third step in CoA biosynthesis from pantothenate. The K_M for this reaction is 800 μ M for PPC.

Subsequently, it was established that Dfp is a bifunctional enzyme, catalyzing the second step of CoA biosynthesis, coupling of 4'-phosphopantothenate with

cysteine to form PPC, as well. This reaction is a two-step process and requires CTP for initial 4'-phosphopantothenate activation. Second step couples cysteine to the phosphopantothenate moiety with a release of CMP. Estimated K_M 's are 300 μ M for 4'-phosphopantothenate and CTP, and 250 μ M for cysteine.

5

CoaBC as target for antibacterial development.

Coenzyme A (CoA) plays a vital role in the metabolism of living cells. According to a recent report, 4% of all enzymes in the cell require CoA, its thioesters or 4'-phosphopantetheine. Recent genetic footprinting experiments on *E. coli* and direct gene knockout have established that this *coaBC* is essential for bacterial growth. Homologs of *coaBC* have been identified in a number of gram-positive and gram-negative organisms, which suggested the possibility of developing a broad-spectrum antibacterials from *coaBC* inhibitors. Considering the bifunctional nature of CoaBC, it is feasible to identify inhibitors that will inhibit both enzymic functions, thus arresting two steps in the CoA pathway. Another important factor in favor of selecting CoaBC as a target for antibacterials is low homology of the bacterial enzyme to eukaryotic counterparts. In most of the higher organisms including humans, two separate enzymes carry out these functions. Moreover, mammalian (R)-4'-phospho-N-pantothenoylcysteine decarboxylase is a pyruvate-dependent enzyme, while CoaBC requires flavine mononucleotide for its function.

15

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Assays for measuring CoaBC activity.

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PPC synthetase activity is be monitored by detecting the released pyrophosphate. This is achieved by converting pyrophosphate to inorganic phosphate with pyrophosphatase and detection by the Malachite Green assay, or by the MESG assay spectrophotometrically. CoaBC (2 μ g) is incubated in the reaction buffer containing 10 mM DTT, 2 mM $MgCl_2$, 50 mM Tris-HCl, pH 8, 300 μ M 4'-phosphopantothenate, 3.5 mM CTP, 5 μ g pyrophosphatase. The reaction is started by addition of appropriate amount (10-500 μ M final) of cysteine. The reaction is stopped at different time points by addition of equal volume of 5M H_2SO_4 . The

amount of inorganic phosphate released will be determined according to the one of described techniques.

PPC synthetase activity is also monitored by detecting the release of carbon dioxide from ^{14}C -labeled cysteine. CoaBC (2 μg) is incubated in the reaction buffer containing 10 mM DTT, 2 mM MgCl_2 , 50 mM Tris-HCl, pH 8, 2.5 μM 4'-phosphopantothenate, 3.5 mM CTP. The reaction is started by addition of appropriate amount (30 mM, final concentration) of ^{14}C -labeled cysteine. The reaction is stopped at different time points by addition of equal volume of 5M H_2SO_4 . Amount of released ^{14}C -labeled CO_2 is determined according to published technique.

Example 8

Identification of the gene encoding phosphopantetheine adenylyltransferase (CoaD) in *Alloiococcus otitidis*

Phosphopantetheine adenylyltransferase, (PPAT, CoaD, KdtB) catalyzes the penultimate step in Coenzyme A (CoA) biosynthesis. The fourth step in CoA biosynthesis is the addition of AMP to 4'-phosphopantetheine by phosphopantetheine adenylyltransferase (CoaD) to form 3' dephospho-CoA (dPCoA).

The *coaD* gene was first identified in *E. coli* by Geerlof *et al.* CoaD is essential for viability in *E. coli* and *S. aureus*. The enzyme has a mass of 18 kDa and was determined to be a hexamer through cross-linking studies. Crystallography confirmed the oligomeric state of the enzyme. Moreover, co-crystallography of CoaD with dPCoA has also been carried out mapping the binding pocket for the major product of the reaction. Interestingly, in mammals PPAT has been shown to be in a complex with dephospho Coenzyme A kinase (dPCoA kinase, DPCK). This enzyme, purified from pig liver, is referred to as CoA Synthase. The yeast PPAT is associated with a protein complex that is in excess of 375 kDa and composed of six proteins. There is no detectable homology between the bacterial PPAT (CoaD) and the recently identified human PPAT, the activity of which is contained in a bifunctional PPAT/DPCK enzyme. Homologues of *E. coli* CoaD have been identified in *P. aeruginosa*, *S. pneumoniae*, *S. aureus*, *H. influenzae*, *H. pylori*, *B. anthracis* and *M. tuberculosis*. Homologue of this gene identified in *Alloiococcus otitidis* as described in

Example 5 (Seq. ID No 81). The protein encoded by the gene is set forth in Seq. ID No. 82.

Enzyme activity

5 CoaD (PPAT) carries out the reversible transfer of AMP to 4'-phosphopantetheine, forming dephosphocoenzyme A and releasing PPi. The reverse reaction was demonstrated by Geerlof *et al.* using a coupled assay to tie ATP production to NADP reduction, which is monitored at 340 nm. The following kinetic constants were calculated: $k_{cat} = 3.3 \pm 0.1$ /sec; $K_{m(dPCoA)} = 7.0 \pm 1.4$ uM;
10 $K_{m(PPi)} = 0.22 \pm 0.04$ mM.

CoaD as target for anti-infective development.

Coenzyme A biosynthesis is essential for bacterial viability. CoaD,
15 phosphopantetheine adenylyltransferase, catalyzes the fourth step in the pathway and was shown to be essential in both *E. coli* and *S. aureus*. There is no measurable homology between CoaD and the human PPAT enzyme, so the liability of poorly selective compounds is quite low. As CoaD is essential and conserved in gram-negative and gram-positive pathogens, inhibitors developed against this target will
20 have broad-spectrum utility.

Assays for measuring CoaD function

CoaD will be expressed and purified using standard methodologies for bacterial expression and affinity tag-based purification. Two assay formats can be
25 used to monitor enzymatic activity: the forward reaction and the reverse reaction.

The forward reaction assay was initially described for measuring the activity of the human PPAT activity in the PPAT/DPCK enzyme. The enzyme assay is carried out in 50 mM Tris (pH 8.0), 2 mM MgCl₂, 5 mM ATP, 5-500 uM 4'-phosphopantotheine, 7.5 mM NADH and enzyme (initially 0.1 – 1.0 µg/ml). The
30 production of PP_i is detected using the protocol of O'Brien in which PP_i production is coupled to the oxidation of NADH to NAD. This system requires the addition of 4 enzymes (PP_i-dependent phosphofructokinase, aldolase, triosephosphate isomerase and glycerol-3-P dehydrogenase) to the basic reaction mix and presents the added issue of deconvolution, which limits the use of the assay as a primary screen.

The reverse direction assay is carried out also as a coupled assay to tie ATP production to NADP reduction following the method described by Lamprecht & Trautschold. The assay is set up in reaction buffer containing the following: 50 mM Tris (pH 8.0), 1 mM DTT, 2 mM MgCl₂, 1 mM NADP, 5 mM glucose, 2 mM PP_i, 0.1 mM dPCoA. Hexokinase (4 units) and glucose-6-phosphate dehydrogenase (1 unit) will be added to the assay as the coupling enzymes in addition to CoaD (initially 0.1 – 1 µg/ml). The assay is monitored at 340 nm. Deconvolution of hits is required with this assay, however with only 2 additional enzymes the task will be less cumbersome when compared to the forward assay described above.

Example 9

IDENTIFICATION OF THE GENE ENCODING DEPHOSPHOCO A KINASE (DPCK, YACE, COAE) IN ALLOIOCOCCUS OTITIDIS

DephosphoCoA kinase (DPCK, YacE, CoaE) encoded by the *coaE* gene catalyzes the final step in Coenzyme A (CoA) biosynthesis. The final step in CoA biosynthesis is the phosphorylation of the 3'-hydroxyl group of dephospho-CoA to form CoA by dephosphocoenzyme A kinase (DPCK, YacE, CoaE).

The determination that the previously identified *yacE* gene encoded the dephosphocoenzyme A kinase activity was reported by Mishra *et al.* These authors previously determined that separate enzymes encode the phosphopantetheine adenylyltransferase (PPAT) and dephosphocoenzyme A kinase (DPCK) activity in *Corynebacterium ammoniagenes* in contrast to the eukaryotic enzymes in which the PPAT and DPCK activities are coupled. The *E. coli* gene, encoding a 25 kDa protein, was cloned based on the sequence of the *C. ammoniagenes* gene and found to be identical to the previously described *yacE* gene. The gene was designated *coaE* to follow existing nomenclature in *E. coli*. *CoaE* (*YacE*) was shown to be essential in *E. coli* through genetic footprinting. *CoaE* is widely distributed in bacteria. Homologue of this gene identified in *Alloioococcus otitidis* as described in Example 5 (Seq. ID No 93). The protein encoded by the gene is set forth in Seq. ID No. 94.

Assays for measuring CoaE function

CoaE carries out the phosphorylation of dephosphocoenzyme A at the 3' hydroxyl group, consuming ATP, to form CoA. Dephosphocoenzyme A kinase activity is measured in a coupled reaction in which NADH oxidation to NAD is tied to ADP production. In this assay, the standard pyruvate kinase/lactose dehydrogenase coupling system is used to generate NAD in a 1:1 molar equivalent to the ADP produced by the test enzyme. NADH oxidation to NAD is monitored at 340 nm in a standard spectrophotometer. The following kinetic values were determined for CoaE: $K_m(\text{ATP}) = 0.74 \text{ mM}$; $K_m(\text{dephospho-CoA}) = 0.14 \text{ mM}$ (7).

The formation of CoA is monitored using a coupled enzyme system in which acetyl-CoA is formed in proportion to the amount of CoA in the assay. Three enzymes (phosphate acetyl transferase, citrate synthase and malate dehydrogenase) are added to the reaction that results in the formation of NADH from NAD, which is monitored at 340 nm.

CoaE as a target for anti-infective development

Coenzyme A biosynthesis is essential for bacterial viability. CoaE, dephosphocoenzyme A kinase, catalyzes the final step in CoA synthesis and is shown to be essential by genetic footprinting in *E. coli*. A degree of homology between CoaE and the human DPCK enzyme has been noted, such that selectivity assays is necessary to determine a high therapeutic index for CoaE inhibitory compounds. CoaE is conserved in gram-negative and gram-positive pathogens and should have broad-spectrum utility in the clinic.

CoaE is expressed and purified using standard methodologies for bacterial expression and affinity tag-based purification. DephosphocoA kinase activity is monitored using a coupled enzyme system to tie ADP production to oxidation of NADH to NAD. The decay of absorbance at 340 nm will be the assay readout. The assay will be setup in the following buffer: 50 mM Tris (pH 8.5), 20 mM KCl, 10 mM MgCl_2 , 10 mM ATP, 0.3 mM NADH and 0.4 mM phosphoenolpyruvate. The coupling enzymes: pyruvate kinase (10 U) and lactate dehydrogenase (4 U) will be added along with dephosphocoenzyme A kinase (initially 0.1- 1.0 ug/ml). The assay will be started by the addition of 0.4 mM dephosphocoenzyme A. In this assay system, the release of ADP is tied to the oxidation of NADH to NAD, and is monitored at 340 nm.

This assay is transferable to a high-density microtiter plate format and suitable for HTS.

EXAMPLE 10

5 IDENTIFICATION OF DNAB AND PCRA, GENES ENCODING HELICASES IN ALLOIOCOCCUS
OTTIDIS

Helicases unwind double-stranded DNA in a reaction that couple nucleotide binding and hydrolysis to strand unwinding. Their activity is required for a number of biological processes such as separation of the chromosome during replication, recombination and repair. Homologue of these genes were identified in *Alloiococcus otitidis* as described in Example 5 (Seq. ID No 15 and 99). The protein encoded by the gene is set forth in Seq. ID No. 16 and 100.

15 Due to the essential roles modulated by these molecules they represent an important target for antibacterial therapy. Homologs of *dnaB* and *pcrA* genes encoding helicases were identified as described in Example 5. A primary assay, which detects helicase function *in vitro*, is used to identify inhibitors of each enzyme and is described below.

20 Genes encoding DnaB and PcrA is obtained using polymerase chain reaction amplification of the genomic region encoding them. The genes is subcloned into a standard expression vector either containing an amino acid tag for ease of purification or not. The enzyme is then over-expressed in *Escherichia coli* and purified using a standard tag system.

Most helicases require a region of single-stranded DNA flanking the duplex region that it unwinds. As a result, providing a single stranded region to either the 3' or 5' end of a duplex allows for determination of the polarity of helicase unwinding. These types of experiments have demonstrated that PcrA and DnaB are 3'-5' and 5'-3' helicases, respectively. None the less, a convenient filtration assay has previously been described that is formatted for high-through-put screening of inhibitors of either enzyme, regardless of polarity. Assays (90 ul) contained 15 pM single-stranded M13 DNA to which a radiolabeled oligonucleotide had been annealed as a substrate for unwinding. Reactions are carried out in 96-well GF/C unifilter hydrophobic plates (Polyfiltronics Inc.) in 70 ul helicase buffer [20 mM Hepes (pH 7.6), 4 mM MgCl₂ 4

mM ATP, 100 ug/ml BSA, 5% glycerol and 2 mM DTT] and 10 ul of DMSO or compound. Reactions are initiated by adding 10 ul of purified helicase protein and are incubated for 1 hr at room temperature. 100 ul of 2X capture buffer containing silica beads [25% methanol, 3 M NaI, 0.03% NP-40, and 10% GlassFog beads (BIO101)] were added. The mixture was incubated for 30 min at room temperature. Plates are then washed 5X on a Bio-Tek instruments, Auto Washer EL403) with wash buffer (50% ethanol, 0.2% NP-40 and 50 mM NaCl). Scintillation fluid was added and plates are counted (Packard Topcount).

10

EXAMPLE 11

IDENTIFICATION OF DnaE, THE GENE ENCODING DnaE-POLYMERASE IN *ALLOIOCOCCUS OTITIDIS*

DnaE is an enzyme that catalyzes the DNA template directed polymerization of deoxyribonucleotides into deoxyribonucleic acid. The enzyme has been reported to modulate lagging strand synthesis at gram-positive replication forks. Functions for DnaE have been defined biochemically, in *Bacillus subtilis* and *Streptococcus pyogenes*. Homologue of this gene identified in *Alloiococcus otitidis* as described in Example 5 (Seq. ID No 75). The protein encoded by the gene is set forth in Seq. ID No. 76.

Because DnaE is an essential protein in gram-positive bacteria and has high homology to the gram-negative *dnaE*, which is an essential polymerase subunit of the DNA polymerase III holoenzyme, it serves as a good target for antibacterial drug discovery. A primary assay, which detects processive DnaE mediated DNA synthesis *in vitro*, is useful identify inhibitors of the enzyme and is described below.

The gene encoding DnaE I in *Alloiococcus otitidis* was identified as described in Example 5. Purification of DnaE DNA polymerase from *Alloiococcus*. The gene encoding DnaE is obtained using polymerase chain reaction amplification of the *dnaE* gene. The gene is subcloned into a standard expression vector either containing an amino acid tag for ease of purification or not. The enzyme is then over-expressed in *Escherichia coli* and purified using a standard tag system.

Because DnaE catalyzes the incorporation of single deoxyribonucleotides into DNA, the incorporation of radiolabelled deoxyribonucleotides into larger deoxyribonucleic acid molecules is monitored to measure activity of the enzyme. A

filtration assay has been previously described for *Streptococcus pyogenes* DnaE that uses filterplates containing DE81 filters to capture polymerized DNA. This assay is amenable to high-through-put screening format for DnaE. Assays contained 70 ng of 30-mer primed M13mp18 single stranded DNA as a template for replication. The
5 reaction contained 3.3-300 ng of DnaE in 23.5 μ l of replication buffer [20 mM Tris-HCL (pH 7.5), 4% glycerol, 0.1 mM EDTA, 5 mM DTT, 2 mM ATP, 8 mM $MgCl_2$, 40 μ g/ml BSA] and 60 μ M of both dGTP and dCTP. NaCl was added to the reaction mixture to a final concentration of 40 mM. DNA synthesis was initiated by the addition of 1.5 μ l of 1.5 mM dATP and 0.5 mM [μ - ^{32}P]dTTP. Reactions were
10 incubated at 37°C for various lengths of time and were quenched by adding an equal volume of 1% SDS and 40 mM EDTA. One-half of the terminated reaction was applied to DE81 filter paper and washed 3X with wash solution (0.3 M Ammonium formate and 0.01 M Sodium pyrophosphate). Filters were then placed in scintillation vials and 1 ml scintillation counting liquid was added. Radioactivity was counted
15 using a scintillation counter.

EXAMPLE 12

IDENTIFICATION OF DNAG, THE GENE ENCODING PRIMASE IN *ALLOIOCOCCUS OTITIDIS*

20 DnaG is an enzyme that catalyzes the DNA template directed polymerization of ribonucleotides into ribonucleic acid *de novo*. Ribonucleic acid molecules that are synthesized by DnaG primase subsequently serve as primers for synthesis of the leading- and lagging-strands during chromosomal replication. Functions for DnaG have been defined biochemically, and the crystal structure of the RNA polymerase
25 domain has been determined in *Escherichia coli*. Homologue of this gene identified in *Alloiococcus otitidis* as described in Example 5 (Seq. ID No 63). The protein encoded by the gene is set forth in Seq. ID No. 64.

Because DnaG primase plays an essential role in both leading- and lagging-strand synthesis during chromosomal replication, and DnaG has homologs in all
30 prokaryotes but not eukaryotes, it serves as a good target for antibacterial drug discovery. A primary assay, which detects DnaG mediated RNA synthesis *in vitro*, can be used to identify inhibitors of the enzyme and is described below.

Assay for the activity of DNA polymerase and identification of compounds that inhibit DnaG

The gene encoding DnaG is obtained using polymerase chain reaction
5 amplification of the *dnaG* gene. The gene is subcloned into a standard expression
vector either containing an amino acid tag for ease of purification or not. The
enzyme is then over-expressed in *Escherichia coli* and purified using a standard tag
system.

Because DnaG catalyzes the incorporation of single ribonucleotides into RNA,
10 the incorporation of radiolabelled ribonucleotides into larger ribonucleic acid
molecules is monitored to measure activity of the enzyme. A high-throughput
scintillation proximity assay (SPA) assay, previously described for *E. coli* DnaG, is
used to measure activity of DnaG activity in a coupled reaction with DnaB helicase.
The assay, which was shown to work with DnaG alone, is used to screen for
15 compounds that inhibit DnaG function. Assays are run in 96-well Packard Optiplat
plates. First, 1 μ l DMSO or test compound was added, followed by 20 μ l of DnaG
(208 nM) and 3.3 nM M13mp18 single-stranded DNA. Reactions are initiated by
adding 10 μ l of primase assay buffer [50 mM Tris-HCl (pH 7.5), 4% sucrose, 8 mM
DTT, 5 mM $MgCl_2$, 40 μ g/ml BSA, 0.1 μ g/ μ l Rifampicin, 25 U/ml RNA guard, 100 μ M
20 GTP, 100 μ M UTP, 3 μ M CTP, 1 mM ATP] and 0.4 μ Ci [3H]CTP. Reactions are
incubated at 30°C for 30 min. Next, a suspension of 50 μ l of 2.5 mg/ml PVT-PEI
SPA beads (Amersham; prepared in 0.3 M NaCitrate, pH 3.0) were added. Plates
were read after 1 hr on a Topcount instrument (Packard).

25

EXAMPLE 13

DNAN, DNAX, HOLA, HOLB, AND POLC, THE GENES ENCODING THE SUBUNITS OF
ALLOIOCOCCUS OTITIDIS DNA POLYMERASE III HOLOENZYME: BETA (β), TAU (τ), DELTA
30 (Δ), DELTA' (Δ') AND POLC.

30

DNA polymerase III holoenzyme is an enzyme complex comprised of multiple
highly conserved subunits that catalyzes the DNA template directed polymerization of
deoxyribonucleotides into deoxyribonucleic acid. In gram positive organisms the
holoenzyme is composed of a polymerase subunit, PolC, and accessory proteins.
35 The accessory proteins act in a coordinated manner to clamp the polymerase tightly
to the DNA template allowing the polymerase to synthesize DNA with high speed and

processivity. Homologue of these genes identified in *Alloiococcus otitidis* are described in Example 5 (Seq. ID Nos. 21, 105, 79, 103, and 105 respectively). The protein encoded by the gene is set forth in Seq. ID No. 22, 106, 80, 104 and 106 respectively).

5 Functions for the individual subunits have been defined biochemically and interactions between them have now been deduced structurally by crystallographic analysis of the enzyme from *Escherichia coli*. Tau interacts directly with both delta and delta' to form a clamp loader complex. Upon binding ATP the complex undergoes a conformational change altering an interaction between delta and delta',
10 which allows delta to subsequently interact with the beta-clamp. The beta-clamp is a ring-shaped homomultimer assembly that can be opened by delta and placed onto a primed DNA template. ATP hydrolysis results in closing the clamp around DNA and dissociation of the clamp-loading complex. PolC then couples with the beta clamp to form a highly processive polymerase.

15 Because DNA polymerase III holoenzyme is comprised of multiple subunits, the opportunity exists to inhibit its activity at a number of different sites. A primary assay, which detects processive DNA synthesis *in vitro*, can be used to identify inhibitors of the enzyme and is described below. Deconvolution of inhibitors, based on either activity of physical interaction, follow the primary assay.

20

Assay for the activity of DNA polymerase

Purification of DNA polymerase III holoenzyme subunits from *Alloiococcus*. Genes encoding the subunits of DNA polymerase is obtained using polymerase chain reaction (PCR) amplification of the genomic region encoding them. The genes
25 are subcloned into a standard expression vector either containing an amino acid tag for ease of purification or not. The enzyme is then over-expressed in *Escherichia coli* and purified using a standard tag system.

Because DNA polymerase III catalyzes the incorporation of single deoxyribonucleotides into DNA, the incorporation of radiolabeled deoxynucleotides
30 into larger deoxyribonucleic acid molecules is monitored to measure activity of the enzyme. A filtration assay is previously described for *Streptococcus pyogenes* DNA polymerase III that uses filterplates containing DE81 filters to capture polymerized DNA (2). This assay is amenable to high-through-put screening format. Assays

contained 70 ng of 30-mer primed M13mp18 single stranded DNA as a template for replication. The reaction contained 43 ng of β and 140 ng of PolC- τ complex in 23.5 μ l of replication buffer (20 mM Tris-HCL (pH 7.5), 4% glycerol, 0.1 mM EDTA, 5 mM DTT, 2 mM ATP, 8 mM $MgCl_2$, 40 μ g/ml BSA, and 60 μ M of both dGTP and dCTP. DNA synthesis was initiated by the addition of 1.5 μ l of dATP and [μ - ^{32}P]dTTP. Reactions were incubated at 37°C for various lengths of time and were quenched by adding an equal volume of 1% SDS and 40 mM EDTA. One-half of the terminated reaction was applied to DE81 filter paper and washed 3X with wash solution (0.3 M Ammonium formate and 0.01 M Sodium pyrophosphate). Filters were then placed in scintillation vials and 1 ml scintillation counting liquid was added. Radioactivity was counted using a scintillation counter.

Compounds inhibiting PolC subunit is identified by modifying the above reaction to include only the PolC subunit and using 2.5 μ g activated calf thymus DNA as a substrate, instead of singly-primed M13mp18 DNA, as previously described. Several techniques are utilized to determine the interaction of inhibitors with individual subunits. These have been described in the literature and include the following: (1) Nuclear magnetic resonance and capillary electrophoresis.

EXAMPLE 14

ERA GTPASE IN ALLOIOCOCCUS OTITIDIS

The *era* (*E. coli* Ras) gene was initially identified while sequencing around the *rnc* gene; *era* lies downstream of *rnc*. While a function for *era* has yet to be determined, conditional (temperature sensitive) mutants revealed that the product of the *era* gene, Era, is essential for *E. coli* viability. A hint as to an *in vivo* function for Era was uncovered when a suppressor of a *dnaG* (primase) allele was found to map in the *era* coding sequence and a second suppressor, which mapped upstream of the *era* open reading frame, affected expression of *era*. These data suggest that Era could play one or more roles in DNA replication, regulation of primase activity or otherwise effect cell cycle progression. More recent data has confirmed that the *era1* mutant causes a defect in cell growth at the two-cell stage and delays cell division. Moreover, Britton et al demonstrated that cell division was coupled with the level of

Era in the cell: division arrest, through reduction in Era levels, is reversed when Era levels return to threshold amount. A current model suggests that Era acts as a checkpoint regulator in the bacterial cell cycle. Era is a GTP-binding protein with GTPase activity, a threshold level of functional/activated Era may be required to initiate septation.

Era is associated with additional cellular functions, specifically translation, as Era specifically interacts with the translation machinery. *E. coli* Era binds both 16S rRNA and the 30S ribosomal subunit; whereas, the *S. pneumoniae* 16S rRNA co-purifies with Era. A putative RNA binding "KH motif" has been identified in the carboxyl-terminal domain. The RNA binding activity is critical to Era cellular function as mutation of the putative RNA binding region of the *S. pneumoniae* Era prevents complementation of an *E. coli era* mutant strain. Homologue of this gene identified in *Alloiococcus otitidis* as described in Example 5 (Seq. ID No 65). The protein encoded by the gene is set forth in Seq. ID No. 66.

Nucleotide binding

Filter-binding assays are utilized to demonstrate nucleotide-binding specific to GTP and not UTP, CTP or ATP. Both GTP and GDP (unlabeled) were capable of inhibiting $\alpha^{32}\text{P}$ -GTP binding. The Kd for GTP and GDP binding were reported to be 5.5 and 1.0 μM , respectively.

A large number of GTP-binding proteins have been studied and all members of the family contain three regions of highly homologous amino acid residues that define a GTP-binding pocket. Era contains well-conserved regions defining the so-called G1 (G/AXXXXGKT/S: residues 15-22), G3 (DXXG: residues 62-65) and G4 (NKXD: residues 124-128) consensus sequences. The G2 domain (residues 33-38, see below), located between G1 and G3, is generally more variable.

GTPase activity

Purified Era showed a significant GTPase activity, which is inhibitable by GTP or GDP but not by UTP, CTP, ATP or ADP. The maximum hydrolysis rate is measured at 9.8 mmol GTP hydrolyzed/min/mol Era. The Km was found to be 9 μM .

It should be noted that Sullivan *et al* demonstrated, using mant (*N*-methyl-3'-O-anthraniloyl) labeled GTP and GDP, very rapid exchange kinetics for guanine

nucleotide binding. Era exchanges guanine nucleotides 10-fold more rapidly than the GTP hydrolysis rate suggesting that guanine nucleotide binding and release should be considered as a regulatory point in addition to the more well-studied hydrolysis step.

5

Autophosphorylation

When $\gamma^{32}\text{P}$ -GTP is used as a substrate for the GTPase activity, Era is phosphorylated. The autophosphorylation reaction is specific for GTP, as incubation with $\gamma^{32}\text{P}$ -ATP did not result in phosphorylation of Era. Moreover, $\alpha^{32}\text{P}$ -GTP is not a
10 suitable substrate for detection of Era autophosphorylation. Tryptic digestion and HPLC were utilized to resolve the sites(s) of phosphorylation. Using $\gamma^{32}\text{P}$ -GTP as a substrate the major radioactive peak contained the tryptic peptide, ISITSR, corresponding to Era residues 33-38 and containing 3 potential phosphorylation sites. Mutagenesis of both Thr-36 and Ser-37 to alanine abolished enzymatic
15 activity. However, individual alanine substitutions at either site had no effect on Era function. The autophosphorylation site is located in the so-called G2 domain of Era.

Suitability of target for anti-infective development

Era is an essential protein for bacterial viability. Knock-down mutations as well
20 as conditional-lethal alleles revealed that Era function is required for cytokinesis. An additional phenotype of the Era-depleted strains is an aberrant response to temperature induced stress. This target is novel and may well lead to the identification of new classes of anti-infectives. The widespread distribution of Era homologues in both gram-negative and gram-positive pathogens suggests that
25 broad-spectrum agents could result from an effort to define Era inhibitory compounds.

Assays for measuring Era function

30 NUCLEOTIDE BINDING ASSAYS

Era binding to nucleotide is monitored by a simple filter-binding assay. Era (1-5 μg) is incubated with $\alpha^{32}\text{P}$ -GTP (0.2 μCi) in a buffer consisting of 100 mM Tris (pH 7.5), 10 mM MgCl_2 , 0.2% NP-40, 0.2 mg/ml BSA for 30 minutes at 32°C. A

portion of the reaction mix is spotted on nitrocellulose membrane, washed (50 mM Tris (pH 7.5), 5 mM MgCl₂, 1 mM DTT) and dried. The membrane is then exposed to X-ray film. Alternatively, the spots are excised and counted. This assay is directly amenable to HTS using filter plates.

5

GTPASE ACTIVITY ASSAY

The GTP hydrolytic activity of Era is monitored using thin-layer chromatography. Era and $\alpha^{32}\text{P}$ -GTP is incubated in 50 mM Tris (pH 7.5), 5 mM MgCl₂, 0.1% NP-40, 0.2 mg/ml BSA for 30 minutes at 37°C. An aliquot of the
10 reaction is placed on PEI cellulose and the strip developed with 0.5 M KH₂PO₄, 1.0 M NaCl (pH 3.7). The spots conforming to GDP and GTP are identified by UV shadowing, excised and counted. This assay represents an acceptable secondary/confirmatory assay.

Alternatively, the hydrolysis of $\gamma^{32}\text{P}$ -GTP is monitored by assaying for
15 liberated P_i. Obg and $\alpha^{32}\text{P}$ -GTP is incubated in 50 mM Tris (pH 8.5), 1.5 mM MgCl₂, 0.1 mM EDTA, 100 mM KCl, 10% glycerol for 30 minutes to 3 hours at 37°C. The reaction will be stopped by the addition of a slurry of charcoal in 1 mM Kpi (pH 7.5), which selectively binds the GTP and GDP. The liberated P_i in the supernatant is monitored by Cerenkov counting. Free P_i can also be monitored with the Malachite
20 Green reagent.

AUTOPHOSPHORYLATION ASSAY

Era autophosphorylation is monitored by incubating Era with $\gamma^{32}\text{P}$ -GTP in 50 mM morpholinopropane sulphate (pH 6.8), 5 mM MgCl₂, 1 mM DTT at 37°C (14).
25 Samples are analyzed following separation on SDS polyacrylamide gels, drying the gel and exposure to film. This assay represents an acceptable secondary/confirmatory assay for Era activity.

EXAMPLE 15

FMHB(FEMX) GENES IN ALLOIOCOCCUS OTITIDIS

5 The *femA*, *femB*, and *fmhB(femX)* genes have been shown to be essential for incorporation of glycine into the side chain of peptidoglycan precursors in *Staphylococcus aureus*. The *femAB* locus was initially identified as a factor essential for methicillin resistance (*fem*) based on random insertional inactivation of chromosomal genes and a screen for reduced expression of resistance mediated by
10 the penicillin binding protein 2A (PBP2A). Inactivation of *femA* or *femB* was subsequently reported to prevent incorporation of glycine residues at positions 2 to 5 or positions 4 to 5 of the penta-glycine cross bridge since mucopeptides cross-linked by one or three glycine residues were detected in the corresponding mutants. Inactivation of *fmhB*, formerly *femX*, is lethal, but the construction of a mutant
15 conditionally expressing *fmhB* under the control of a xylose-inducible promoter showed that the gene was essential for synthesis of branched peptidoglycan precursors. These studies show that the *fem* gene products were required for incorporation of glycine at positions 1 (FmhB), 2 and 3 (FemA), and 4 and 5 (FemB) of the cross bridge, although the catalytic activity of the proteins has not been directly
20 assessed. Similarly, inactivation of two *fmhB* homologues in *Streptococcus pneumoniae*, designated *murM (fibA)* and *murN (fibB)*, reduced addition of L-Ala or L-Ser to the -amino group of L-Lys and subsequent addition of a second L-Ala residue, respectively. Overall, disruption of the *murMN* operon reduced the proportion of branched peptide stems in the peptidoglycan from 89 to 33%. In contrast to what
25 occurs in *S. aureus*, direct cross-linking of L-Lys to D-Ala occurs in *S. pneumoniae*, and the *murMN* operon was accordingly reported to be unessential.

 BLAST analysis of *Alloiococcus otitis* genome revealed an ORF similar to *femX* of *Weissella viridescens*, and *fmhB* of *S. aureus*. It suggests that in *Alloiococcus otitis* there is an enzyme with similar to FmhB function. Homologue of
30 this gene identified in *Alloiococcus otitidis* is described in Example 5 /Table 4 (Seq. ID No 97). The protein encoded by the gene is set forth in Seq. ID No. 98.

Assays for measuring FmhB function

There are no *in vitro* biochemical assays to test enzymatic activity of *S. aureus* FmhB because the reaction occurs at the membrane-bound lipid II precursor GlcNAc-(β -1,4)-*N*-acetylmuramic acid(-L-Ala-D-iGln-L-Lys-D-Ala-D-Ala)-pyrophosphoryl-undecaprenol.

Lipid II is a minor component of bacterial cell membrane which is detected by thin-layer chromatography separation of presolubilized membranes supplied with the cytoplasmic precursors, UDP-*N*-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) and [14 C]UDP-*N*-acetylglucosamine ([14 C]UDP-GlcNAc).

The *in vitro* biosynthesis of branched lipid II of *S. aureus* requires whole-cell membranes, cytoplasmic PG precursors, glycine (14 C labeled for detection of reaction products), purified tRNA, and an intracellular fraction that contains tRNA-activating enzymes. Therefore, the *in vitro* assay of *S. aureus* FmhB is a tedious procedure.

One way to facilitate this procedure is to use *Weissella viridescens* FemX or *E. faecalis* UDP-MurNAc-pentapeptide:L-alanine ligase. Recombinant *Weissella viridescens* FemX and *E. faecalis* UDP-MurNAc-pentapeptide:L-alanine ligase were purified, and their *in vitro* activity was demonstrated. The distinctive feature of these enzymes is that they catalyze the addition of a branching amino acid (Ala) to the cytoplasmic cell wall precursor UDP-MurNAc-pentapeptide.

Other bacteria for which the biosynthesis of Gly-containing branched UDP-MurNAc-hexapeptide in cytoplasm was shown are *Streptomyces lividans* and *Streptomyces hydroscopicus*, although the enzymes were not isolated and their ligase activity remain to be demonstrated.

These new data open an opportunity to develop an assay to detect the activity of FmhB(FemX) by using cytoplasmic UDP-MurNAc-pentapeptide. Products of the reaction are detected by HPLC. HPLC separation of precursors are performed by the method of Flouret et al. The precursors are separated by reverse-phase HPLC on a μ Bondapak C₁₈ column (3.9 by 300 mm; Waters) in 50 mM ammonium formate (pH 3.9) at a flow rate of 0.5 ml/min. The elution of precursors is monitored at a wavelength of 254 nm.

EXAMPLE 16

FOLA- DIHYDROFOLATE REDUCTASE (DHFR)

5 The *Alloicoccus* ORF-1863 encodes a homolog of *S. aureus* dihydrofolate reductase that catalyzes the NADPH-dependent conversion of dihydrofolate to tetrahydrofolate, one of the steps in bacterial folate biosynthesis. Homologue of this gene identified in *Alloicoccus otitidis* is described in Example 5/Table 4 (Seq. ID No 55). The protein encoded by the gene is set forth in Seq. ID No. 56.

10

FOLA as a target for anti-infective development

Folate is an essential cofactor in many important metabolic processes in bacteria, such as purine, pyrimidine, amino acid and pantothenate biosynthesis. Unlike mammalian cells, bacteria are unable to utilize exogenous folate derivatives, and therefore must synthesize folate *de novo*. Bacterial folate biosynthesis occurs via two converging pathways, the non-essential para-amino-benzoate (PABA) synthesis pathway, and synthesis of the pterin precursor, to which pABA is subsequently attached to form the folate precursor. Bacterial DHFRs are essential for viability and well conserved across all bacterial species. Although bacterial DHFR shares

15 similarity with human DHFR, selective inhibitors against bacterial DHFR have been identified in the past such as trimethoprim which specifically blocks the bacterial DHFR step. Thus DHFR still remains an attractive target for development of broad-spectrum antibacterial agents.

25 **Assays for measuring DHFR activity**

DHFR activity is monitored spectrophotometrically, recording the change of absorbance at 340 nm due to the equimolar consumption of NADPH in the course of dihydrofolate substrate reduction. DHFR (10 ng) is preincubated in reaction buffer containing 50 mM 2-(N-morpholino)ethanesulfonic acid, 25 mM Tris-HCl, 25 mM

30 ethanolamine, and 100 mM NaCl at pH 7.5 for 3 minutes. The reaction is started by addition of 0.5-10 μ M 7,8-dihydrofolate. The amount of processed substrate is calculated from the decrease of absorbance at 340 nm due to oxidation of NADPH ($\epsilon=11800 \text{ M}^{-1}\text{cm}^{-1}$) to NADP^+ .

EXAMPLE 17**FOLB- DIHYDRONEOPTERIN ALDOLASE (DHNA)**

5 The *Alloiococcus otitidis* ORF-959 encodes a homolog of *S. aureus* dihydroneopterin aldolase that catalyzes the conversion of 7,8-dihydroneopterin to 6-hydroxymethyl-7,8-dihydropterin, one of the early steps in bacterial folate biosynthesis. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 31). The protein encoded by the gene is set forth in
10 Seq. ID No. 32.

FOLB as a target for anti-infective development

Folate is an essential cofactor in many important metabolic processes in bacteria, such as purine, pyrimidine, amino acid and pantothenate biosynthesis.
15 Unlike mammalian cells, bacteria are unable to utilize exogenous folate derivatives, and therefore must synthesize folate *de novo*. Bacterial folate biosynthesis occurs via two converging pathways, the non-essential para-amino-benzoate (*p*ABA) synthesis pathway, and synthesis of the pterin precursor, to which *p*ABA is subsequently attached to form the folate precursor. Enzymes that catalyze steps in the folate
20 biosynthesis pathway are essential and well conserved across all bacterial species, and those that act in early steps such as FolB have no direct homologs in mammals. Thus FolB becomes an attractive target for development of broad-spectrum antibacterial agents.

25 Assays for measuring FOLB activity

FolB (DHNA) 7,8-dihydroneopterin aldolase activity is monitored individually or in conjunction with downstream enzymes in folic acid biosynthesis pathway (FolK and Sul).

FolB activity is monitored directly by HPLC assay. FolB substrate (7,8-dihydro-D-neopterin) is commercially available from Schircks Laboratories
30 (Switzerland). FolB (0.5 µg) is preincubated in reaction buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.1 mg/ml BSA, 2.5 mM dithiothreitol for 5 min. Reaction is started by addition of stock solution of 7,8-dihydro-D-neopterin in DMSO (100 µM

final concentration). Reaction is terminated by addition of 1/3 of reaction volume of 1% I₂, 2% KI in 1M HCl with subsequent incubation at room temperature for 5 minutes. Quenched reaction will be applied directly to HPLC. Oxidized starting material and reaction products are efficiently separated on ODS (C18) column.

- 5 Reaction components are detected and quantified by analysis of UV absorbance at 254 nm, or fluorescence (excitation at 365 nm; emission at 446 nm).

FolB activity are also monitored in the coupled assay with FolK (HPPK) and Sul (DHPS) enzymes. FolB activity is measured by detection of radioactive dihydropteroate formation as described in FolK and Sul assays, under conditions of
10 excess of the later enzymes. FolB enzyme and substrate 7,8-dihydro-D-neopterin are added to the described assay to replace the 6-hydroxymethyl-7,8-dihydropterin (FolK substrate).

EXAMPLE 18

15 FOLC- DIHYDROFOLATE SYNTHASE (DHFS)

The *Alloiococcus otitidis* ORF-956 and ORF-528 both encode a homolog of *B. subtilis* dihydrofolate synthase that catalyzes the conversion of 7,8-dihydropteroate and glutamate to dihydrofolate, one of the steps in bacterial folate biosynthesis [.

- 20 Homologue of this gene identified in *Alloiococcus otitidis* as described in Example 5 (Seq. ID Nos. 29 and 23). The protein encoded by the gene is set forth in Seq. ID Nos. 30 and 24.

Use of FOLC as a target for anti-infective development

- 25 Folate is an essential cofactor in many important metabolic processes in bacteria, such as purine, pyrimidine, amino acid and pantothenate biosynthesis. Unlike mammalian cells, bacteria are unable to utilize exogenous folate derivatives, and therefore must synthesize folate *de novo*. Bacterial folate biosynthesis occurs via two converging pathways, the non-essential para-amino-benzoate (*pABA*) synthesis
30 pathway, and synthesis of the pterin precursor, to which *pABA* is subsequently attached to form the folate precursor. Enzymes that catalyze steps in the folate biosynthesis pathway are essential, and are well conserved across all bacterial species. Bacterial FolC appears to be a bifunctional enzyme possessing both

dihydrofolate synthase (DHFS) activity and folyl-polyglutamate synthetase (FPGS) activity, which are probably mediated through different sites of the protein. The bacterial DHFS activity but not the FPGS activity is essential for viability. Although bacterial FolC shares similarity with human FPGS, the human enzymes apparently lack DHFS activity and display a folate substrate specificity quite distinct from that of bacterial enzymes. Thus targeting bacterial FolC/DHFS activity selectively might lead to identification of broad-spectrum antibacterial agents.

Assays for measuring FOLC activity

FolC (DHFS) 7,8-dihydrofolate synthase activity in the presence or absence of antimicrobial compounds or putative inhibitory compounds are monitored by several methods.

In one method, FolC activity is monitored directly by simple HPLC assay. FolC substrate (7,8-dihydropteroic acid) is commercially available from Schircks Laboratories (Switzerland). FolC (15 ng) is added to reaction mix, containing 10 mM glutamate, 5 mM ATP, 50 mM Tris-HCl (pH 8.0), 20 mM Mg₂Cl, 50 mM KCl, 0.1 mg/ml BSA, 5 mM dithiothreitol. Reaction is started by addition of stock solution of 7,8-dihydropteroic acid in DMSO (10 μ M final concentration). Reaction is terminated by addition of equal volume of 8M Guanidinium hydrochloride. Stopped reaction is applied directly to HPLC. Starting material and reaction products are efficiently separated on ODS (C18) column. Reaction components are detected and quantified by analysis of UV absorbance at 254 nm, or fluorescence (excitation at 280 nm; emission at 420 nm).

In another method, the FolC activity monitoring is by detection of ADP accumulation. ADP is released in the amount equimolar to the amount of the product formed. ADP detection is performed by coupling its conversion to ATP by pyruvate kinase in the presence of phospho(enol)pyruvate producing pyruvate. Lactate dehydrogenase reduces pyruvate to S-lactate in the presence of NADH. Course of reaction is monitored by decrease in absorbance at 340 nm due to oxidation of NADH ($\epsilon=6220 \text{ cm}^{-1}\text{M}^{-1}$) to NAD⁺. Reaction conditions are as following: 5 mM dithiothreitol, 5 mM ATP, 380 μ M NADH, 10 mM glutamate, 2 mM phospho(enol)pyruvate, 50 mM KCl, 20 mM Mg₂Cl, 50 mM Tris-HCl, 50 μ g of

pyruvate kinase, 50 µg of S-lactate dehydrogenase. Reaction is started by addition 7,8-dihydropteroic acid in DMSO (10 µM final concentration).

In yet another method, FolC activity is monitored through detection of inorganic phosphate release. Amount of inorganic phosphate in solution is quantified by:

(i) its conversion by purinenucleoside phosphorylase leading to phosphorylation of MESG. Later assay kit is available from Molecular Probes as EnzCheck™ Phosphate Assay Kit;

(ii) its reaction with Malachite Green reagent; and

(iii) detecting the release of radioactive inorganic phosphate in reaction with γ-³³P-labeled ATP following the absorption of unprocessed ATP by charcoal.

First method is applied in rate-based assay format; the later two in end-point format. Reaction conditions are similar to the ones described in HPLC-based assay.

EXAMPLE 19

FOLK- 6-HYDROXYMETHYL-7, 8-DIHYDROPTERIN PYROPHOSPHOKINASE (HPPK)

The *Alloiococcus otitidis* OFR-961 (Seq. ID No. 33) encodes a homolog of *S. aureus* 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase that catalyzes pyrophosphoryl transfer from ATP to 6-hydroxymethyl-7,8-dihydropterin, one of the early steps in bacterial folate biosynthesis. The protein encoded by this ORF is set forth in Seq. ID No. 34. (see Example 5/Table 4).

Use of FolK as a target for anti-infective development

Folate is an essential cofactor in many important metabolic processes in bacteria, such as purine, pyrimidine, amino acid and pantothenate biosynthesis. Unlike mammalian cells, bacteria are unable to utilize exogenous folate derivatives, and therefore must synthesize folate *de novo*. Bacterial folate biosynthesis occurs via two converging pathways, the non-essential para-amino-benzoate (*p*ABA) synthesis pathway, and synthesis of the pterin precursor, to which *p*ABA is subsequently attached to form the folate precursor. Enzymes that catalyze steps in the folate biosynthesis pathway are essential and well conserved across all bacterial species, and those that act in early steps such as FolK have no direct homologs in mammals.

Thus FolK is an attractive target for the development of broad-spectrum antibacterial agents.

Assays for measuring FolK activity

5

FolK (HPPK) 7,8-dihydroxymethylpterin-pyrophosphokinase activity is monitored individually or in conjunction with downstream enzyme in folic acid biosynthesis pathway.

10 FolK activity is monitored directly by HPLC assay. FolK substrate (7,8-dihydro-6-hydroxymethylpterin) is commercially available from Schircks Laboratories (Switzerland). FolK is preincubated in reaction buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM KCl, 20 mM MgCl₂, 5 mM ATP, 0.1 mg/ml BSA, 2.5 mM dithiothreitol. Reaction is started by addition of stock solution of 7,8-dihydro-6-hydroxymethylpterin in DMSO (100 μM final concentration). Reaction is terminated by addition of equal
15 volume of 8M Guanidinium hydrochloride and applied directly on HPLC. Starting material and reaction products are efficiently separated on ODS (C18) column. Reaction components are detected and quantified by analysis of UV absorbance at 254 nm.

FolK activity is monitored by end-point assay coupled with excess of Sul enzyme.
20 Activity is calculated from quantification of the radioactivity incorporated in final product (7,8-dihydropteroate).

EXAMPLE 20

ALLOIOCOCCUS OTITIDIS ENCODED FOLP (SUL)- DIHYDROPTEROATE SYNTHASE (DHPS)

25

The *Alloiococcus otitidis* ORF-1811 (Seq. ID No. 53) encodes a homolog of *B. subtilis* dihydropteroate synthase that catalyzes the condensation of pABA (para-aminobenzoic acid) with 6-hydroxymethyl-7,8-dihydropterin pyrophosphate, one of the early steps in bacterial folate biosynthesis. The polypeptide encoded by this ORF
30 is set forth in Seq. ID No. 54. (see Example 5/Table 4)

FOLP AS A TARGET FOR ANTI-INFECTIVE DEVELOPMENT

Folate is an essential cofactor in many important metabolic processes in bacteria, such as purine, pyrimidine, amino acid and pantothenate biosynthesis. Unlike

mammalian cells, bacteria are unable to utilize exogenous folate derivatives, and therefore must synthesize folate *de novo*. Bacterial folate biosynthesis occurs via two converging pathways, the non-essential para-amino-benzoate (*p*ABA) synthesis pathway, and synthesis of the pterin precursor, to which *p*ABA is subsequently
5 attached to form the folate precursor. Enzymes that catalyze steps in the folate biosynthesis pathway are essential and well conserved across all bacterial species, and those that act in early steps such as FolP (Sul) have no direct homologs in mammals. In fact, dihydropteroate synthase (FolP or Sul) is the target for known antibiotics sulfonamides which are competitive inhibitors of FolP/Sul as *p*ABA
10 analogues. Thus FolP (Sul) still remains an attractive target for development of broad-spectrum antibacterial agents.

Suitable assays for measuring FolP/Sul activity

Sul (DHPS) 6-hydroxymethyl-7,8-dihydroneopteroate synthase activity is
15 monitored individually or in conjunction with upstream enzymes in folic acid biosynthesis pathway (FolB and/or FolK).

DHPS activity is monitored directly by counting the amount of radioactivity incorporated in 6-hydroxymethyl-7,8-dihydroneopteroate when using radioactively
20 labeled *p*-aminobenzoic acid (*p*ABA). Final product is separated from unreacted *p*ABA by thinlayer chromatography, paper chromatography or on HPLC equipped with radioactivity detector. DHPS substrate (6-hydroxymethyl-7,8-dihydropterin pyrophosphate) is not commercially available, but is quantitatively synthesized in one step from its oxidized precursor available from Schircks Laboratories (Switzerland). DHPS (20 ng) is added in reaction buffer containing 50 mM Tris-HCl, pH 8.0, 20 mM
25 MgCl₂, 0.1 mg/ml BSA, 5 mM dithiothreitol and 0.5 – 10 μM PABA. Reaction is started by addition of stock solution of substrate (6-hydroxymethyl-7, 8-dihydropterin pyrophosphate, 0.05 - 1 μM final concentration). Reaction is terminated by acidification of reaction volume with addition of equal volume of citrate/phosphate or ammonium acetate/acetate buffer, pH 4 containing excess of unlabelled *p*ABA.
30 Quenched reaction is separated by chromatography and the amount of formed product calculated.

DHPS activity is determined in coupled assay with excess of FolB and FolK enzymes. The advantage of coupled assay is that it makes it possible to use

commercially available FolB (7,8-dihydro-D-neopterin), or FolK (6-hydroxymethyl-7,8-dihydropterin) substrates, thus forming DHPS substrate *in situ*.

EXAMPLE 21

5 ALLOIOCOCCUS OTTIDIS ENCODED FILAMENTATION TEMPERATURE SENSITIVE GENE A
(FtsA)

The *Alloiococcus otitidis* ORF-2489 (Seq. ID No. 85) encodes a homolog of *E. faecalis* FtsA, one of the essential components of bacterial cell division. The “fts” stands for filamentation temperature sensitive and has been assigned to most bacterial cell division genes due to the fact that these genes were generally discovered by the isolation of conditional mutants that form filaments at nonpermissive temperature . The *ftsA* allele was first isolated and identified in *E. coli* by Ricard and Hirota in 1973, and mapped along with *ftsZ* in 1980. The protein encoded by this ORF is set forth in Seq. ID No. 86. (see Example 5/Table 4).

Bacterial cell division requires formation of a septum at mid-cell that begins with the polymerization of FtsZ into a ring structure at the nascent division site. FtsZ, another key component of bacterial septation is the first known protein to localize to the division site. In *E. coli*, shortly after the formation of the FtsZ ring, FtsA and ZipA (another key division component present only in gram-negative bacteria) [7] are independently recruited to the septal ring, most likely through their direct interaction with FtsZ. Subsequent assembly of other division components at the septum requires FtsA as well as FtsZ.

25 **FtsA as a target for anti-infective development**

Like FtsZ, FtsA homologs are present and highly conserved in almost all eubacteria. FtsA is essential for cell division and its deletion leads to impaired cell division and sporulation defect. In addition, *E. coli* cells have to maintain critical ratio of FtsA to FtsZ in order for proper cell division to occur. FtsA belongs to the actin/DnaK/sugar kinase family of proteins. In *B. subtilis*, FtsA acting as a dimer not only binds ATP but also hydrolyzes ATP. As briefly stated above, *in vivo* and *in vitro* evidence have demonstrated that FtsA and FtsZ from various bacterial species

directly interact. Taken all together, targeting at FtsA especially at its interaction with FtsZ might lead to identification of broad-spectrum antibacterial agents.

Assays for measuring FtsA activity

5

ATPase activity of FtsA is assayed by following the formation of $^{32}\text{P}\text{i}$ from $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$. The reaction mixture containing 50 mM Tris-HCl (pH7.2), 50 mM potassium acetate, 1 mM DTT, 10 mM MgCl_2 and different concentrations of $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ is incubated for 5 minutes at 37°C . The reaction is started by addition of 50 nM purified
10 FtsA of *Alloicoccus*. The reaction is stopped with 1.5% ammonium molybdate in 0.5N sulfuric acid, and the radioactive Pi extracted into isoamyl alcohol and counted.

Interaction between FtsA and FtsZ is detected quantitatively using yeast two-hybrid system as described. Briefly, *Alloicoccus ftsZ* is cloned into yeast two-hybrid bait vector pLexA (Clontech) to generate a LexA-FtsZ fusion with DNA-binding
15 property. *Alloicoccus ftsA* is cloned into the target vector pB42AD (Clontech) to fuse FtsA to the activating domain. Both plasmids are then transformed into a *Saccharomyces cerevisiae* strain containing a *lacZ* reporter under the control of multiple LexA operators. β -Galactosidase activity is determined to quantify relative strength of FtsA-FtsZ interaction.

20

EXAMPLE 21

ALLOIOCOCCUS OTITIDIS ENCODED FILAMENTATION TEMPERATURE SENSITIVE GENE Z (FtsZ)

25

FtsZ is an essential protein that forms a cytokinetic ring (Z-ring) that drives cell division in bacteria. FtsZ has been identified in most prokaryotic species with the exception of *Chlamidia*, a *Ureaplasma* species and *Crenarchaea*. FtsZ and Z-ring formation are most extensively studied in *E. coli*. FtsZ is an abundant cytoplasmic protein which is present at $\sim 10^4$ copies per cell, and is the first protein to be localized
30 to the division site. Z-ring is required throughout septation and directs the ingrowth of septum in part by recruiting other cell division protein to the division site. Another function is suggested by FtsZ homology to eukaryotic tubulins. Like tubulin, FtsZ is a GTPase and undergoes GTP/GDP-dependent polymerization. Recent studies showed that Z-ring is a very dynamic structure suggesting that GTP-dependent

assembly/disassembly of Z-ring might provide constriction force to power cell division. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 83). The protein encoded by the gene is set forth in Seq. ID No. 84.

5

GTPase activity

FtsZ is a GTPase that contains the tubulin-signature nucleotide-binding motif GGGTGS/TG. Like in $\alpha\beta$ -tubulin dimer, the active site for GTP-hydrolysis appears to be shared between two subunits where the GTP-binding pocket is provided by one subunit while the GTPase-activating T7 loop comes from the other subunit. This view is supported by genetic analysis as various mutations that inhibit FtsZ GTPase activity map in the T7-loop region and a conserved Asp-residue in T7-loop is found to be involved in the coordination of the cation involved in GTP hydrolysis. FtsZ GTPase activity is Mg^{2+} -dependent and is stimulated by KCl.

15

Polymerization

In vivo, about 75% of FtsZ is present as multimers. *In vitro*, FtsZ forms a variety of structures at various conditions. FtsZ assembles into thin protofilaments with GTP and formation of FtsZ polymers is coupled to GTP hydrolysis: when GTP runs out, polymers disassemble. Protofilaments assemble into sheets and bundles in the presence of multimolar amounts of either Mg^{2+} or Ca^{2+} or by addition of DEAE-dextran. In addition, ZipA protein induces bundling of FtsZ polymers. With GDP, FtsZ assembles into curved filaments and minirings.

25

Interactions with other proteins

In *E. coli*, at least nine different proteins are localized to the division septum and are required for cell division to proceed. Among them two proteins, ZipA and FtsA, are shown to interact directly with FtsZ. Both of these proteins localize to the division site independently from each other, but require FtsZ for localization. ZipA is an integral membrane protein which is thought to mediate invagination of cell membrane by linking the membrane to constricting Z-ring. Interaction between ZipA and FtsZ is confined to C-terminal portion of ZipA (residues 185-328) and conserved 17-amino acid region on C-terminus of FtsZ. FtsA is an actin-like membrane-associated protein

30

which possesses ATPase activity and might provide energy required for Z-ring dynamics. Interaction between FtsZ and FtsA is not studied in great detail, it is shown that C-terminus of FtsZ is required. The remaining division proteins require both ZipA and FtsA for their localization to Z-ring.

5

FtsZ as a target for anti-infective development

FtsZ is an essential protein for cell division/bacterial viability. Knock-out *ftsZ* mutants fail to divide and, as a result, filament and die. The target is widely
10 conserved throughout bacterial kingdom implying that FtsZ-specific inhibitor would have a broad-spectrum antibacterial activity. The potential drawbacks of the target might include the presence and the essential role of a homolog (tubulin) in eukaryotes and an intrinsic difficulty in inhibiting protein-protein interactions by small molecules. Although this target is being studied extensively, no FtsZ-specific
15 compounds are reported up to date.

Assays for measuring FtsZ function

Polymerization of FtsZ is measured by light scattering assay as described previously. FtsZ (12.5 μ M) is incubated in 200 μ l of polymerization buffer (50 mM
20 MES/NaOH, pH 6.5, 50 mM KCl, 5 mM MgCl₂, 10 mM CaCl₂) in a fluorescence cuvette with a 1 cm path length. The sample is maintained at 30°C, polymerization is induced by addition of 20-500 μ M GTP. Light scattering is measured at 90°, both excitation and emission wavelengths are set to 350 nm, slit width is 2 nm.

Alternatively, the amount of polymerized FtsZ is analyzed by sedimentation and
25 subsequent quantification of precipitated FtsZ by SDS-PAGE, Coomassie staining and densitometric scanning. In addition, polymers are observed by electron microscopy. This assay represents either primary or secondary/confirmatory assay.

GTP binding of FtsZ is monitored by the covalent cross-linking of [γ -³²P]GTP (3000 Ci/mmol) to FtsZ in a previously described competition assay. FtsZ (3 μ g) is
30 incubated in 20 μ l of 50 mM MES/NaOH, pH 6.5, 100 mM KCl, 4 mM MgCl₂, 1 mM EDTA, 0.1 mM EGTA and 0.5 mM DTT. Various amounts of non-labeled competing nucleotide (GTP or GTP analogs) and 0.1 mM [γ -³²P]GTP are added, samples are incubated at 0°C for 15 min, then UV cross-linked for 5 min and analyzed by SDS-

PAGE on 12% gel, autoradiography and densitometric scanning. This assay represents a secondary/confirmatory assay.

The GTP hydrolytic activity of FtsZ is monitored by thin-layer chromatography (TLC) as described previously. Briefly, the reaction mixture consists of 5 mM of [γ - 32 P]GTP (40 mCi/mmol), 15 mM magnesium acetate and 0.25-2 mg/ml of FtsZ in reaction buffer (40 mM Tris-acetate, pH7, 200 mM potassium acetate, 2 mM EDTA, 1 mM DTT and 0.5% Triton X-100), aliquots are separated by TLC and amount of GTP converted to GDP is determined by spot-densitometry. Alternatively, GTPase activity is measured either by quantitation of the non-radioactive inorganic phosphate with the malachite green-molybdate reagent as described previously or by quantitation by scintillation counting of radioactive inorganic phosphate released after hydrolysis of [γ - 32 P]GTP (26). This assay represents either primary or secondary/confirmatory assay.

Among interactions of FtsZ with various cell division proteins, interaction between FtsZ and ZipA is characterized the best. ZipA -induced bundling of FtsZ is measured by the light scattering assay that is described above, both proteins are used at ≥ 5 μ M.

EXAMPLE 22

ALLOIOCOCCUS OTITIDIS ENCODED GYRA/GYRB (DNA GYRASE, TOPOISOMERASE II) AND GRLA/GRLB (TOPOISOMERASE IV)

DNA topoisomerases: topoisomerases modulate the topological state of DNA in cells. This involves binding to DNA, introducing single or double stranded breaks in the DNA, passing DNA molecules through the break and rejoining the break. This controls the levels of positive and negative supercoiling of DNA and functions in catenation/decatenation. Controlling the topological state of DNA is critical to the fundamental processes of transcription, recombination, replication and partitioning of the chromosome. There are two main categories of topoisomerases, type I and type II. Type I topoisomerases introduce single stranded breaks in DNA whereas type II enzymes introduce double stranded breaks. GyrA/GyrB (gyrase) and GrlA/GrlB (topoisomerase IV) are both type II enzymes that are essential for cell viability.

DNA gyrase (GyrA/GyrB) is a type II topoisomerase that functions to control the degree of supercoiling in double stranded DNA. It is essential for viability and

plays central roles in replication, repair, recombination and transcription of DNA. Gyrase have the ability to introduce double stranded breaks in DNA molecules while remaining bound to the DNA through phosphotyrosine bonds, pass uncut DNA through the break and then rejoin the breaks, with repeated cycles being driven by the hydrolysis of ATP. Gyrase has the unique ability to introduce negative supercoils in closed circular DNA and also functions to catenate/decatenate DNA duplexes. The generation of negative supercoiling is important for initial stages in replication. DNA gyrase from *Escherichia coli* has been studied in detail. It is a complex of two subunits of GyrA (encoded by *gyrA*) and two subunits of GyrB (encoded by *gyrB*) (ie. A_2B_2 complex). The subunits are organized in discrete domains. An N-terminal domain of GyrB harbors ATPase activity while the C-terminal domain is thought to interact with the GyrA subunit, and is involved in DNA binding. The N-terminal domain of GyrA is apparently involved in DNA strand breakage-ligation reactions while the C-terminal segment is involved in DNA binding. Crystal structures of the DNA strand breakage/reunion domain of *E. coli* GyrA, and the N-terminal ATPase domain of *E. coli* GyrB have been determined. DNA gyrase has also been purified and characterized from gram positive organisms such as *S. aureus*. Comparison of DNA gyrases from several bacteria reveal a high degree of conservation of important domains.

Topoisomerase IV (GrlA/GrlB) is a type II topoisomerase but unlike gyrase it does not possess negative supercoiling activity. Its primary role in replication appears to be in the decatenation of multiply linked daughter chromosomes, important for terminal stages of the replication process. Topoisomerase IV has been purified and characterized from gram negatives eg. *E. coli*, (where the GrlA/GrlB subunit homologs are designated ParC and ParE), and gram positives eg *S. aureus*. Homologs of these genes identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID Nos 17 and 19). The proteins encoded by the genes are set forth in Seq. ID Nos. 18 and 20.

GyrA/GyrB (Gyrase) and GrIA/GrIB (topoisomerase IV) as targets for anti-infective development:

Alloiococcus otitidis is an infectious organism associated with disease, and consequently, novel antimicrobials to combat these infections are desirable. DNA gyrase and Topoisomerase IV is essential for bacterial viability and is a well-established and validated antibacterial target.

Purification of DNA gyrase and topoisomerase IV from *Alloiococcus otitidis*

Genes encoding the GyrA/GyrB and GrIA/GrIB subunits or their functional domains are obtained using polymerase chain reaction amplification of the genomic region encoding them. The genes are then subcloned into standard expression vectors, with or without affinity tags. The enzyme is then overexpressed in *Escherichia coli* and purified using a standard tag system or conventional chromatography.

Measurement of gyrase and topoisomerase IV by kinetoplast DNA decatenation assay:

Type II topoisomerases introduce double stranded breaks in DNA and mediate catenation/decatenation of DNA. Topoisomerase IV activity is readily determined with decatenation assays using as substrate kinetoplast DNA (KDNA) from *Crithidia fasciculata*. The DNA isolated in this procedure is a highly networked series of catenated double stranded minicircles and is easily be pelleted by centrifugation. The activity of topoisomerase II enzymes results in the release of decatenated DNA minicircles from the networked KDNA. These have a high mobility in agarose gels and migrate into the gel ahead of the networked material, which has very low mobility, allowing for determination of decatenation activity using ethidium bromide stained agarose gel electrophoresis.

Alternatively, using radiolabeled KDNA, the level of decatenation activity is measured by counting radioactivity remaining in reaction supernatants following centrifugation to pellet the networked material. Typical conditions used for assaying decatenation activity of *S. aureus* and *E. coli* topoisomerase IV activity are as follows: *C. fasciculata* KDNA (0.9 mg/ml) is incubated in 40 µl of reaction buffer (50 mM Tris-HCl, pH 7.7, 5 mM MgCl₂, 5 mM DTT, 50 µg/ml bovine serum albumin, 1.5 mM ATP

and 350 mM potassium glutamate) with appropriate amounts of the Grl subunits, for 1 hour at 37° C. If non radiolabeled KDNA is used, these reactions can be stopped and analyzed by agarose gel electrophoresis, or for radioassays, the reaction is stopped by gentle mixing with 10 µl of stop solution (50 % glycerol, 50 mM EDTA (pH 8.0), 2.5 % SDS and 0.1 % bromphenyl blue) and centrifuged at 15 000 x g for 5 min at 20° C. Decatenation activity is determined by counting radioactivity in 25 µl of the supernatant in a scintillation counter. Alternatively, a modified assay employing flow injection fluorometry of 4', 6-diaminidino-2-phenylindole (DAPI) treated supernatants has been described that could be suitable for moderate throughput non radioactive assays, or filtration of the reactions through appropriate filters may efficiently separate the decatenated species from KDNA. Although the above described assays were used for topoisomerase IV, modified decatenation reactions using KDNA isolated from *Leishmania donovani* reveal significant decatenation activity by gyrase from *E. coli* and *Mycobacterium smegmatis*, indicating the applicability of the assay to prokaryotic gyrases.

DNA Supercoiling/relaxation assays.

DNA gyrase function is directly assayed using a simple supercoiling assay typified by that described for the measurement of *Escherichia coli* DNA gyrase activity. Briefly, incubation of relaxed closed circular plasmid DNA (pUC18, 7.5 nM) in the presence of DNA gyrase (approximately 10 nM) in 40 mM Tris-HCl (pH 8.0) buffer containing 25 mM KCl, 4 mM MgCl₂, 2.5 mM spermidine and 1.4 mM ATP buffer results in the introduction of supercoils in the plasmid DNA. Changes in DNA supercoiling status are readily observed by the alteration of mobility of the DNA in agarose gels stained with ethidium bromide and comparison to the mobility of relaxed and supercoiled plasmid template. This strategy is employed for screening for DNA gyrase inhibitors.

Topoisomerase IV activity is assayed by measuring relaxation of supercoiled plasmid DNA. A typical relaxation assay used for *S. aureus* topoisomerase IV activity is as follows: topoisomerase IV enzyme and supercoiled plasmid DNA (pBR322, 0.6 µg) is incubated in 40 µl 50 mM Tris-HCl, pH 7.7, containing 5 mM MgCl₂, 5 mM DTT, 50 µg/ml bovine serum albumin, 1.5 mM ATP, 5 mM spermidine and 20 mM KCl, for 30 min at 37°C. Changes in DNA supercoiling status can be

readily observed by the alteration of mobility of the DNA in agarose gels stained with ethidium bromide and comparison to the mobility of relaxed and supercoiled plasmid template

The ATPase activity of topoisomerases is measured using a coupled
5 spectrophotometric ATPase assay described for the GyrB subunit of *E. coli*. ATPase activity is assayed in 300 μ l of 40 mM Tris-HCl (pH 8.0), containing 25 mM KCl, 2.5 mM spermidine, 4 mM MgCl₂, 400 μ M phosphoenolpyruvate, 250 μ M NADH, 3 μ l of pyruvate kinase /lactate dehydrogenase mix and ATP (0.5 – 3.5 mM). The reaction is started by the addition of truncated N-terminal derivatives of the GyrB protein (5
10 μ M) containing the ATPase domain. ATPase activity is reflected as a decrease in absorbance of light at 340 nanometer wavelength.

DNA cleavage assay.

Quinolone drugs interfere with the DNA strand breakage-ligation cycle activity
15 of many topoisomerases. Incubation of topoisomerase and linear or supercoiled pBR322 plasmid DNA, or small linear DNA fragments, in the presence of quinolones and magnesium results in the trapping of a complex of topoisomerase, DNA with a double stranded break and the drug. The topoisomerase remains bound to the cleaved DNA, however treatment with a denaturant such as SDS or proteinases
20 remove/degrade the gyrase, releasing the cut DNA. Certain consensus sequences representing preferred cut sites of *E. coli* gyrase in plasmid pBR322 have been identified in template DNA molecules used in these assays. This assay is useful for mode of action studies of inhibitors of gyrase/topoisomerase IV activity and in particular of the strand breakage-ligation function. Cleavage reactions are performed
25 with linear or supercoiled DNA. A typical cleavage reaction using linear DNA to measure cleavage by *E. coli* and *S. aureus* gyrase and topoisomerase IV in the presence of drugs is as follows: gyrase/ topoisomerase IV is incubated in 20 μ l 25 mM Tris-HCl (pH 7.5) containing 0.5 mM EDTA, 0.5 mM DTT, 3 μ g bovine serum albumin per ml, 10 mM MgCl₂, 120 mM KCL, 10 mM ATP, 10 000 dpm of 3' end
30 labeled linear pBR322 plasmid DNA and drug for 1 hour at 37°C. (Note: for *S. aureus*, KCl is replaced with 0.7 M potassium glutamate). Reactions are terminated by adding 5 μ l 2.5% SDS-2.5 mg proteinase K per ml and incubating at 37°C for 30 minute, then adding 5 μ l 30% glycerol-1% SDS-50 mM EDTA-0.05 % bromophenol

blue. Cleavage products are resolved on 1% agarose gels and visualized by autoradiography.

Additional cleavage assays are also used that measure 1) the linearization of supercoiled plasmid DNA (pBR322), with linearization measured using scanning densitometry of DNA species separated on 1 % agarose gels, or 2) the cleavage of small linear DNA molecules of approximately 100 bp encompassing the preferred cleavage sequence 5'- GGCTGGATGGCCTTCCCCAT - 3' from position 990 in plasmid pBR322. In the latter case, the fragment is produced by PCR and radiolabeled with γ -³²P ATP at the 5' end of the top strand. This DNA is incubated with 1.3 pmol DNA gyrase in a total volume of 10 μ l 35 mM Tris-HCl (pH 8.0), 24 mM KCl, 2 mM spermidine, 4 mM MgCl₂ and inhibitor compound at 37°C for 10 min. Reactions are stopped by addition of 8 mM EDTA and 1% SDS, then treated with 500 μ g/ml proteinase K for 2 hours at 37°C. The DNA is then cleaned by phenol-chloroform extraction and ethanol precipitation, resuspended in TE buffer (pH 8.0), and loaded and resolved on 12 % sequencing gels containing 7M urea. In the presence of inhibitors of the strand breakage-ligation function, radioactive cleavage products are detectable by autoradiography. Modifications of this assay whereby one strand of the DNA substrate is labeled with an affinity tag such as biotin and the other is radiolabeled or fluorescently labeled should facilitate rapid separation and detection of cleavage products using streptavidin coated columns or plates, resulting in higher assay throughput.

GYRASE ACTIVITY ASSAYS: DNA REPLICATION:

Early work by Fuller and Kornberg revealed that a partially purified crude soluble fraction derived from *Escherichia coli* cells (designated fraction II) contained the components necessary for replication of plasmids containing oriC (*E. coli* chromosomal origin of replication). Replication mediated by this fraction specifically required supercoiled plasmids. Although the exact makeup of the protein complex mediating the replication was not known, the replication reaction was inhibited by 1) rifampicin, and 2) nalidixic acid and novobiocin, indicating essential roles for both RNA polymerase and DNA gyrase, respectively. Subsequently the reaction was reproduced using replication machinery reconstituted from purified protein HU, DnaA,

DnaC, DnaB, single stranded binding protein (SSB), primase, DNA polymerase holoenzyme, RNA polymerase holoenzyme and GyrA/GyrB.

The requirement for gyrase activity for replication is exploited for the identification of gyrase inhibitors using a replication-based high throughput screen.

- 5 Gyrase specific inhibitors are identified from the overall pool of replication inhibitors using the secondary assays detailed below. Screening for inhibitors of gyrase in a setting where gyrase is participating in an overall reaction that is essential in bacteria might better select physiologically relevant inhibitors

- 10 An assay suitable for high throughput screening of inhibitors of replication (including gyrase and DnaA inhibitors) is based on the replication reaction of Kaguna and Kornberg. This reaction was set up as follows; standard reaction in 25 μ l: 40 mM Hepes (pH 7.6), 2 mM ATP, 0.5 mM GTP, CTP and UTP, 50 μ g/ml bovine serum albumin, 6 mM phospho creatine, 100 μ M dATP, dGTP, dCTP and dTTP, γ -³³P dTTP (50-150 cpm/pmol total nucleotides) 11mM magnesium acetate, 100 μ g/mL creatine
15 kinase, 85 ng SSB, 48 ng DnaB, 40 ng DnaC, 20 ng primase, 160 ng DNA polymerase III holoenzyme, 800 ng RNA polymerase, 150 ng GyrA, 350 ng GyrB, 120 ng DnaA, 2.5 units topoisomerase I, 190 ng HU, 0.15 ng Rnase H 200 ng supercoiled plasmid template. The reaction is assembled at 0 °C and initiated by incubation at 30°C. Replication reactions are terminated by the addition of EDTA to
20 20 mM. Incorporation of nucleotides into DNA is measured by filtration through 96 well DEAE filter plates and counting retained radioactivity.

- Compounds inhibiting gyrase activity in *Alloicoccus otitidis* are found as part of a larger program directed at replication. This reaction described above uses the replication machinery of a gram-negative organism, which differs somewhat from the
25 replication machinery of gram positives such as *Staphylococcus aureus* with respect to the specific protein subunits involved. Therefore a similar system specific to *Alloicoccus otitidis* is assembled from the relevant proteins purified from *Alloicoccus otitidis*. Several techniques are then utilized to determine the interaction of inhibitors with Gyr A and GyrB. These are described in the literature and include
30 a) Nuclear magnetic resonance; and b) Capillary electrophoresis.

Example 23

ALLOIOCOCCUS OTITIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYMES MUR A

Bacterial cell wall peptidoglycan (murein) is a large macromolecule of periodic
5 structure whose basic unit, a disaccharide-peptide, is polymerized linearly via
the disaccharide motif and cross-linked laterally via the peptide motif. The process of
bacteria cell wall biosynthesis starts from the transferase MurA, which transfers the
addition of an enolpyruvyl moiety to the 3'-hydroxyl-UDP-N-acetyl glycosamine
(UDP-GluNAc). Subsequently, the reductase MurB reduces the enol ether to the
10 lactyl ether, utilize one equiv. of NADPH and a solvent proton to form UDP-N-acetyl
muramic acid (UDP-MurNAc). Next a series of ATP dependent amino acid ligases
(MurC, MurD, MurE and MurF) catalyze the stepwise synthesis of the pentapeptide
side chain using the newly synthesized carboxylate as the first acceptor site. Each
enzyme is responsible for the addition of one more residue except MurF, catalyzes
15 D-ala-D-ala. MurE in gram negative bacteria catalyzes the meso-2, 6-
diaminopimelate (DAP), while in gram positive bacteria MurE catalyzes L-lysine.

The product of MurF, UDP-NAM pentapeptide is the final product of the
cytoplasm enzymes and is the most important precursor for further peptidoglycan
biosynthesis. UDP-MurNAc pentapeptide is then and catalyzed at the plasma
20 membrane by the membrane bound enzymes such as the translocase MraY and
transferase MurG.

UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) catalyzes the first
committed step in bacterial cell wall biosynthesis. The enzyme transfers an
enolpyruvyl group from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine
25 (UDP-GluNAc) to the 3'-OH of UDP-GlcNAc by an addition-elimination mechanism
that proceeds through a tetrahedral ketal intermediate. MurA product enolpyruvate
UDP-N-acetylglucosamine (EP-UNAG) is a precursor to UDP-N-acetylmuramate
(UDP-MurNAc), an essential building block for the bacterial cell wall. MurA is
conserved across both gram-positive and gram-negative bacterial species: gram-
30 negative bacteria have one copy of the *murA* and gram-positive bacteria have two
copies. *Alloiooccus otitidis murA* was identified as described in Example 5/Table 4
and its genomic structure set forth in Seq. ID No. 101. The amino acid sequence of
the protein encoded by this gene is set out in Seq. Id No. 102.

Alloiococcus otitidis murA as a target for anti-infective development

MurA in *E. coli* and *Streptococcus pneumoniae* has been shown to be essential by gene deletion technique. The essentiality of *MurA* in gram-positive bacteria such as *Streptococcus pneumoniae* was demonstrated in that its deletion is fetal. No mammalian homolog to *MurA* has been reported. *MurA* is specifically inhibited by the natural product antibiotic fosfomycin. Thus the importance of *MurA* in peptidoglycan biosynthesis makes it an attractive target for the design of novel antibacterial agent.

10 Assays for measuring MurA function**Phosphate detection:**

MurA activity is detected by quantitating the UDP-GluNAc-dependent Pi from PEP and assayed by Lanzetta's malachite Green-ammonium molybdate assay. Pi is quantitated by measuring the optical density at A660 nm.

Coupled assay with MurB:

A coupled assay in access of MurB, which reduces the *MurA* product EP-UNAG G to UDP-MurNAc, couples the *MurA* transferase activity with NADPH oxidation. The oxidation of NADPH is monitored at 340 nm and is stoichiometric with the production of EP-UNAG.

Fluorescence experiments

Fluorescence experiments to detect *murA* are performed using the hydrophobic fluorescence probe 8-anilino-1-naphthalene sulfonate (ANS). The fluorescence quenching of *MurA*/ANS solutions upon addition of UDP-GlcNAc or pyruvate-P is concentration dependent and in a saturating manner.

Isothermal titration calorimetry

The binding of UDP-GluNAc to *MurA* is studied in the absence and presence of the antibiotic fosfomycin by isothermal titration calorimetry. Fosfomycin binds covalently to *MurA* in the presence of UDP-GluNAc and also in its absence as

demonstrated by MALDI mass spectrometry. Novel Fosfomycin analogs and other antibiotics that bind to *murA* are also identifiable using isothermal titration chemistry.

Capillary electrophoresis-based enzyme assay

5 A capillary electrophoresis-based enzyme assay for MurA is described by Dai and colleagues . This method, based on UV detection, provides baseline separation of one of the reaction products, EP-UNAG, from substrates PEP and UDP-GlcNAc within 4 min. The other product, phosphate, is not detectable by UV at 200 nm. Quantitation of individual components, substrates or product, is be accomplished
10 based on the separated peaks. This assay is also used to detect novel antibiotics, which inhibit *murA* activity.

EXAMPLE 23

ALLOIOCOCCUS OTITIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYMES MURB

15

MurB, the UDP-*N*-acetyl enolpyruvyl glucosamine reductase, commits the second step of bacterial cell wall biosynthesis in cytoplasm and is responsible for the reduction of the enol ether to the lactyl ether, utilizes one equiv. of NADPH and a solvent proton. The product of MurB is UDP-*N*-acetylmuramic acid (UDP-MurNAc), the linker of the peptide
20 and glycan portions of cell wall precursor UDP muramyl-pentapeptide. MurB from *E. coli* is a 342 amino acid protein, which has a distinctive yellow color characteristic of bound flavin as its co-factor. The biochemistry characterization and X-ray crystal structure of MurB in *E. coli*, in *Staphylococcus aureus* and *Streptococcus pneumoniae* have been studied extensively. The gene *Alloiococcus oitidis murB* was identified as disclosed as
25 described in Example 5, and is set out in Seq. ID No. 39. The amino acid sequence of the protein encoded by this gene is set out in Seq. ID No. 40.

Alloiococcus oitidis murB as a target for anti-infective development

30

The essentiality and unique function of MurB in prokaryotic cells and the absence of homologue in eukaryotic cells make it an attractive novel antibacterial target. To date, no small molecule inhibitors of MurB have been reported.

Alloiococcus ostitidis ORF-1263 (*murB*) (Seq. ID No. 39) encodes enzyme UDP-N-acetylenolpyruvylglucosamine Reductase (MurB) as shown by sequence homology.

5 **Assays for measuring *MurB* activity**

Spectrophotometric assay monitoring NADPH consumption:

 MurB activity is typically monitored by its biochemical reaction in which NADPH reduces the bound FAD and resulting decrease in absorbance at 340 nm. Enzyme is maximally activated in the presence of K⁺, NH⁴ at cation concentrations
10 between 10-50 mM.

Coupled assay with *MurC*:

 In designing an end point assay for high through put screen (HTS), a novel coupled assay in access of UDP-MurNAc L-alanine synthase (*MurC*) was developed
15 at Wyeth. This assay utilizes the biochemically synthesized *MurA* product EP-UNAG as substrate, coupled with limited *MurB* and excess *MurC* in the reaction with all other substrates/components involved. In this assay, *MurB* is responsible for the reduction of the enol ether to the lactyl ether, and the follow up enzyme *MurC* catalyzes the ATP dependent ligation of the first of the five amino acids of UDP-
20 peptapeptide with a release of one molecule of phosphate. After 60 minutes of incubation, color reagent malachite green was added and phosphate was detected spectrophotometrically.

Fluorescence binding assay

25 A fluorescence method developed at Wyeth is used to determine the binding potency (K_d value), stoichiometry and nature of binding site of substrates and inhibitors interactions with *MurB* enzymes. This assay is based on changes in intrinsic fluorescence of inhibitor and/or enzyme, upon formation of enzyme-inhibitor complex. Oxidized form of *MurB* consists of two fluorescent groups, namely
30 tryptophan residues and the cofactor FAD. Upon binding inhibitor or substrate, local changes in the solvent environment of these groups or overall conformational and electronic changes occur in the enzyme due to which the fluorescence emission is altered. For instance, inhibitor binding significantly quenched the fluorescence and

altered the solvent environment of FAD to a less polar environment. The changes in the fluorescence of the FAD moiety are used to estimate binding constants for *MurB* inhibitors. Binding experiments are set up in which a fixed concentration of enzyme is titrated with increasing concentrations of the inhibitor. In typical inhibitor binding
5 experiments, the fluorescence emission of the FAD moiety is quenched due to specific interactions of the inhibitor with *MurB* enzymes and the binding site was saturated at micromolar concentrations of inhibitor. The changes in the fluorescence are fitted to mathematical binding models to determine binding affinity.

10 **Temperature-jump isothermal denaturation procedure**

Temperature-jump isothermal denaturation procedure with various methods of detection is used to evaluate the quality of putative inhibitors of *MurB* discovered by high-throughput screening. Three optical methods of detection-ultraviolet
15 hyperchromicity of absorbance, fluorescence of bound dyes, and circular dichroism-as well as differential scanning calorimetry are used to dissect the effects of two chemical compounds and a natural substrate on the enzyme. The kinetics of the denaturation process and binding of the compounds detected by quenching of flavin fluorescence are used to quantitate the dose dependencies of the ligand effects.

20 **NMR studies**

NMR studies are performed using perdeuterated, uniformly $^{13}\text{C}/^{15}\text{N}$ -labeled samples of *MurB*. In the case of substrate-free *MurB*, one or more backbone atoms are assigned for 334 residues (96%). For NADP $^{+}$ -complexed *MurB*, one or more
25 backbone atoms are assigned for 313 residues. The strategies used for obtaining resonance assignments are known. Localizing the NADP $^{+}$ binding site on the *MurB* enzyme is also studied by NMR methodology.

EXAMPLE 25

ALLOIOCOCCUS OTITIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYME, *MURC*

30

Uridine diphosphate-N-acetylmuramate:L-alanine ligase (*MurC*) catalyzes the third chemical step of bacterial cell wall biosynthesis. This enzyme is a nonribosomal peptide ligase which utilize ATP to form an amide bond between L-alanine and UDP-

N-acetylmuramic acid (UDP-MurNAc). This ATP-dependent ligation adds the first of five amino acids to the sugar moiety of the peptidoglycan precursor. Also, in this reaction, ATP is converted to ADP with release of one molecule of inorganic phosphate. Thus MurC reaction is an essential step in cell wall biosynthesis for both gram-positive and gram-negative bacteria. The genetic, biochemistry analysis and crystal graphic studies of MurC in gram-negative bacteria *E. coli* have been extensively studied. Characterizations of MurC in other pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* have also been documented.

10 ***Alloiococcus otitidis* encoded MurC as a target for anti-infective development**

The *Alloiococcus otitidis* ORF-2602 (*murC*, Seq. ID No. 95) encodes enzyme UDP-MurNAc:L-alanine ligase (*MurC*) as determined by sequence homology. This enzyme presents a target for the development of novel anti-infectives to treat the disease(s) caused by this pathogen. Novel compounds identified using combinatorial chemistries are assayed for their inhibitory effect on *MurC* activity using one of the assays set out below.

Assays for measuring MurC activity

20 **Spectrophotometric assay detecting phosphate release:**

MurC activity is detected by the inorganic phosphate production. Typically the reaction mixture contains substrates ATP, L-alanine, UDP-MurNAc, DTT, $MgCl_2$ and MurC enzyme. After 20 minutes incubation, the reaction is quenched with the addition of malachite Green-ammonium molybdate for a colored reaction. Absorbance at 660 nm is read 5 minutes after the quench. Absorbance values are converted to concentration of Pi with standard curves using KH_2PO_4 , which is prepared under identical conditions without the enzyme MurC.

Spectrophotometric assay detecting formation of ADP

30 Due to the conversion of ATP to ADP in MurC reaction, the production of ADP is monitored in coupled enzymes spectrophotometrically. In this reaction, in addition to MurC substrate UDP-MurNAc, L-alanine and ATP, NADH, phosphoenolpyruvate, $MgCl_2$ and $(NH_4)_2SO_4$, two other coupled enzymes pyruvate

kinase and lactase dehydrogenase are also presented. Reaction mixtures without ATP and MurC are incubated at 37°C for 10 min before ATP is added for another minute. Reaction is then started by the addition of MurC. The decrease of NADH absorbance at 340 nm is monitored spectrophotometrically. One unit of activity corresponds to 1 μ mol of ADP formed per hour.

L-Alanine radio-labeled assay:

The MurC enzyme activity in this assay is measured as endpoint using ^{14}C -L-alanine and ATP incubated with MgCl_2 , and $(\text{NH}_4)_2\text{SO}_4$ in 100 mM Tris/HCl, pH 8.0. Reaction is initiated by the addition of the catalytic amounts of MurC. Samples of the reaction mixture are then mixed with glacial acetic acid and then stored at 4°C. Remaining ^{14}C -L-alanine is separated from ^{14}C -UDPMurNAc on SCX columns run under vacuum. Quenched reaction samples are supplemented with equilibration buffer and counted using a liquid scintillation counter.

EXAMPLE 26

ALLOIOCOCCUS OTTIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYMES MURD

Bacterial UDP-N-acetylmuramyl-L-alanine:D-glutamate ligase (MurD), a cytoplasmic peptidoglycan biosynthetic enzyme, catalyzes the fourth step of bacterial cell wall biosynthesis. In this reaction, MurD catalyzes ATP-dependent addition of D-glutamate to an alanyl residue of the UDP-N-acetylmuramyl-L-alanine (UDP-MurNAc-L-Ala) precursor, generating the UDP-MurNAc-dipeptide. The formation of a peptide linkage between the amino function of D-glutamate and the carboxy terminus of UDP-N-acetylmuramuamyl-L-alanine is generated through this reaction. The stoichiometric consumption of ATP supplies the energy needed for this peptide bond formation with concomitant generation of ADP and orthophosphate. The *murD* genes were cloned and characterized from gram-positive bacteria of *Staphylococcus aureus* and *Streptococcus pyogenes*, and gram-negative bacteria from *Escherichia coli*, *Haemophilus influenzae*, *Bacillus subtilis*. Structures of MurD from *E. coli* and MurD complexed with its substrate UDP-MurNAc-L-Ala have been solved to 2.0 Å resolution. The role of specific amino acids at the active site of MurD have been extensively studied using the ortholog and paralog amino acid invariants. Homologue

of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 89). The protein encoded by the gene is set forth in Seq. ID No. 90.

Alloiococcus otitidis encoded MurD as a target for anti-infective development

5

Due to its high specificity and essentiality, MurD is an attractive target for the development of novel antimicrobial agents. *Alloiococcus otitidis* ORF-2494, by sequence homology, has been shown to encode enzyme UDP-N-acetylmuramyl-L-alanine:D-glutamate ligase (MurD) (Seq. ID. No. 89). Inhibition of MurD activity is used to identify novel antimicrobial agents.

10

Assays for measuring MurD activity

Spectrophotometric assay detecting phosphate release:

15

MurD activity in the presence or absence of a putative inhibitory molecule of MurD is detected by the orthophosphate production in test tube or in 96-well format. Typically the reaction mixture contains substrates ATP, D-glutamine, UDP-MurNAc-L-Ala, DTT, MgCl₂ and MurD enzyme. After 20 minutes incubation, the reaction is quenched with the addition of malachite Green-ammonium molybdate for a colored reaction. Absorbance at 660 nm is read 5 minutes after the quench using Molecular Devices SpectraMax 250 plate reader. Absorbance values are converted to concentration of Pi using orthophosphate standards, which are prepared under identical conditions without the enzyme MurD.

20

25

Spectrophotometric assay for detecting formation of ADP in the presence or absence of a putative inhibitory molecule of MurD:

Due to the conversion of ATP to ADP in MurD reaction, the production of ADP is monitored with coupled enzymes of pyruvate kinase and lactase dehydrogenase spectrophotometrically. In this reaction, in addition to MurD substrate UDP-MurNAc-L-ala and ATP, MgCl₂ and (NH₄)₂SO₄, there is also in significant excess of NADH, phosphoenolpyruvate, and two coupled enzymes pyruvate kinase and lactase dehydrogenase. This protocol monitors ADP formation

30

in the MurD catalyzed reaction, in the presence or absence of a putative inhibitory molecule of MurD, by the decrease of NADH absorbance at 340 nm.

L-Glutamate radio-labeled assay:

5 The MurD enzyme activity in the presence or absence of putative inhibitors of MurD is also measurable using D-¹⁴C- glutamate as an endpoint assay. The reaction mixture contains D-¹⁴C- glutamate UDP-MurNAc-L-Ala, ATP, MgCl₂, (NH₄)₂SO₄ in 100 mM Tris/HCl, pH 8.0. An HPLC assay with online UV and flow scintillation detects the formation of UDP-MurNAc-L-Ala-D-¹⁴C Glu and ADP in each reaction.

10

EXAMPLE 27

ALLOIOCOCCUS OTITIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYME, MUR E

15 The fifth step in the cytoplasmic peptidoglycan biosynthetic is catalyzed by MurE. In this step, the monomer units in the *Escherichia coli* and *Staphylococcus aureus* cell wall peptidoglycans differ in the nature of the third amino acid in the L-alanyl-gamma-D-glutamyl-X-D-alanyl-D-alanine side chain, where X is meso-diaminopimelic acid or L-lysine, respectively. Therefore, MurE from *E. coli* is the UDP-N-acetylmuramoyl-L-alanyl-D-glutamate: meso-diaminopimelic acid ligase, and
20 MurE from *S. aureus* is the UDP-N-acetylmuramoyl-L-alanyl-D-glutamate: L-lysine ligase. Thus represents the major difference of MurE from other murein enzymes in cytoplasm. The amino acid residues catalyzed by MurE plays a key role in the integrity of sacculus since it is directly involved in the peptide cross-linkage. MurE reaction is also ATP-dependent, which supplies the energy needed for the peptide
25 bond formation with concomitant generation of ADP and orthophosphate.

30 The essentiality of *MurE* has been well documented in *E. coli*, in *S. aureus*, as well as other pathogens such as *Haemophilis influenzae*, *Vibrio cholerae* and *Corynebacterium glutamicum*. Gene *murE* has been shown to be essential in bacteria. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 25). The protein encoded by the gene is set forth in Seq. ID No. 26.

Alloiococcus otitidis MurE as a target for anti-infective development

Alloiococcus otitidis ORF-851, by sequence homology encodes enzyme UDP-N-acetylmuramyl-L-alanine-D-glutamate ligase: meso-diaminopimelic acid/or L-Lysine (MurE) (Seq. ID No 25). MurE activity in the presence or absence of a putative inhibitory molecule of MurE activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*.

Assays for measuring MurE activity

Radio labeled substrate assay: meso-A2pm-adding activity

Activity of MurE from *Alloiococcus otitidis* in the presence or absence of a putative inhibitory molecule of MurE activity is measured by using radio-labeled meso-¹⁴C A2pm mixing with ATP, MgCl₂, UDP-MurNAc-L-Ala-D-Glu, DTT in 100 mM Tris/HCl and MurE from *Alloiococcus otitidis*.

Radio labeled substrate assay: L-lysine adding activity

Activity of MurE from *Alloiococcus otitidis* in the presence or absence of a putative inhibitory molecule of MurE activity is measured by using radio-labeled UDP-MurNAc-L-Ala-D-¹⁴C-Glu mixing with ATP, MgCl₂, DTT, L-lysine in 100 mM Tris/HCl and MurE from *Alloiococcus otitidis*.

In both cases, mixtures are incubated at 37°C for 30 min, and reactions stopped by the addition of acetic acid. Reaction product is separated by high voltage electrophoresis in 2% formic acid for 45 min. The radio active spots corresponding to substrate and reaction product are detected by overnight autoradiography, or with radio scanner. The spots are also cut out and counted using liquid scintillation counter.

Example 28

ALLOIOCOCCUS OTITIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYME, MURF

The D-alanyl-D-alanine-adding enzyme MurF encoded by the *murF* gene catalyzes is the last step of the cytoplasmic peptidoglycan biosynthesis. MurF performs the ATP-dependent formation of UDP-N-acetylmuramyl-L-gamma-D-Glu-meso-diaminopimelyl-D-Ala-D-Ala (UDP-MurNAc-pentapeptide). The product of MurF, UDP-MurNAc pentapeptide, is the final product of the cytoplasm enzymes and

is the most important precursor for further peptidoglycan biosynthesis. UDP-MurNAc pentapeptide is then catalyzed by the plasma membrane bound enzymes such as the translocase MraY and transferase MurG. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 3). The protein encoded by the gene is set forth in Seq. ID No. 4.

***Alloiococcus otitidis* MurF as a target for anti-infective development**

Due to its high specificity, essentiality, and importance of its product UDP-MurNAc pentapeptide, MurF is attractive as an antibacterial target. The *Alloiococcus otitidis* ORF-48, by sequence homology, encodes enzyme UDP-N-acetylmuramyl-L-alanine-D-glutamate ligase: meso-diaminopimelic acid/or L-Lysine -alanyl-D-alanine-adding enzyme (MurF) (Seq. ID No. 3). MurF activity in the presence or absence of a putative inhibitory molecule of MurF activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*.

Assays for measuring MurF activity

Spectrophotometric assay detecting phosphate release:

Activity of MurF from *Alloiococcus otitidis* in the presence or absence of a putative inhibitory molecule of MurF activity is detected by the inorganic phosphate release in the ATP dependent MurF reaction. This assay detects nonomole amount of Pi in the reaction mixture contains substrates ATP, D-ala-D-ala, UDP-MurNAc-tripeptide, DTT, MgCl₂ and MurF enzyme. After 5 minutes incubation, the reaction is quenched with the addition of malachite Green-ammonium molybdate for a colored reaction.

Coupled spectrophotometric assay detecting formation of ADP

Due to the conversion of ATP to ADP in MurF reaction, the production of ADP in the presence or absence of a putative inhibitory molecule of MurF activity, is monitored with coupled enzymes of pyruvate kinase and lactate dehydrogenase spectrophotometrically. In this reaction, the decrease at 340 nm is observed as NADP is consumed in MurF reaction process. The reaction typically contains tris

buffer, substrates ATP, D-ala-D-ala, UDP-MurNAc-tripeptide, DTT, MgCl₂, phosphoenopyruvate, NADPH and MurF enzyme.

EXAMPLE 29

5 **ALLOIOCOCCUS OTITIDIS ENCODED CELL WALL BIOSYNTHETIC ENZYME, MURG**

MurG, the last enzyme involved in the intracellular phase of peptidoglycan synthesis, is a membrane-associated glycosyltransferase. MurG catalyzes the transfer of *N*-acetyl glucosamine from UDP to the C4 hydroxyl of a lipid-linked *N*-
10 acetyl muramic acid derivative (lipid I) to form lipid II. Lipid II is a linked disaccharide that is the minimal subunit of peptidoglycan. Once lipid II is formed, this disaccharide is translocated across the bacterial membrane where it is polymerized and cross-linked to form the peptidoglycan layers. MurG has been shown to be essential for bacterial survival. The inactivation of MurG gene rapidly inhibits peptidoglycan
15 synthesis in exponential growing cells. As a result, various alterations of cell shape are observed, and cell lysis finally occurs. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 87). The protein encoded by the gene is set forth in Seq. ID No. 88.

20 ***Alloiococcus otitidis* MurG as a target for anti-infective development**

MurG is shown to be associated with the inner face of cytoplasmic membrane, and establishing that the entire peptidoglycan monomer unit assembled before being transferred across the membrane. MurG is a key enzyme at the border
25 line between cytoplasmic and membrane of peptidoglycan synthesis, thus makes it an attractive target for novel antibacterial agent. Further, no mammalian analogues of MurG have been identified. Due to its high specificity, essentiality, and importance, MurG is attractive as an antibacterial target.

The *Alloiococcus otitidis* ORF-2492 has been shown to encode, by sequence
30 homology, glycosyltransferase (MurG) (Seq. ID No.....). MurG activity in the presence or absence of a putative inhibitory molecule of MurG activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*.

Assays for measuring MurG function

Radiolabeled reaction

Activity of MurG from *Alloicoccus otitidis* in the presence or absence of a putative inhibitory molecule of MurG activity is measured by using ^{14}C labeled N-UDP-GluNAc in the reaction containing UDP-MurNAc-pentapeptide, MgCl_2 , ATP and MurG protein. The reaction is stopped after 30 min incubation and by boiling for 3 min. The reaction mixtures are applied to a Whatman I filter paper and subject to descending chromatography overnight. Radioactivity is located and countered with a scanner. This assay is also used to identify the specificity of inhibitor of MraY or MurG, based on the detection of radiolabeled ^{14}C GluNAc incorporated into membrane precursors.

Fluorometric assay

Based on the decrease in NADPH fluorescence at 465 nm, MurG reaction is also monitored in a reaction mixture of HEPES buffer, MgCl_2 , Triton, phosphoenolpyruvate, and coupled enzymes of lactic dehydrogenase and pyruvate kinase, UDP-GluNAc and synthesized lipid I analogue in the presence or absence of putative inhibitors of MurG activity. One micromolar UDP corresponds to 500-fluorescence unit under the instrument setting.

EXAMPLE 30

ALLOIOCOCCUS OTITIDIS ENCODED BY HMG CoA REDUCTASE (MVA A)

Two pathways for isopentenyl diphosphate (IPP) synthesis have been described in bacteria: the mevalonate pathway and the non-mevalonate (MEP or GAP-pyruvate) pathway. The mevalonate pathway predominates in the archaeobacteria, gram-positive organisms, yeast and mammals; whereas the MEP pathway is found in gram-negative organisms, *B. subtilis*, chlamydia, and mycobacterium. The first HMG CoA reductase gene to be sequenced was cloned from *P. mevalonii*, in which HMG CoA reductase permits growth on mevalonate as a sole carbon source. A number of genes of the mevalonate pathway were identified in *S. aureus*, *S. epidermidis*, *S. pyogenes*, *S. pneumoniae*, *E. faecalis* and *E. faecium*. One of the genes, which encodes for HMG-CoA reductase (*mvaA*), when deleted

severely attenuated for virulence in a mouse model indicating that *mvaA* is essential. Due to its high specificity, essentiality, and importance, *mvaA* is attractive as an antibacterial target. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 37). The protein encoded by the gene is set forth in Seq. ID No. 38.

HMG-CoA reductase (MvaA) as a target for anti-infective development

The *Alloiococcus otitidis* ORF- has been shown to encode, by sequence homology, HMG-CoA reductase (*mvaA*) (Seq. ID No 37). *MvaA* activity in the presence or absence of a putative inhibitory molecule of HMG-CoA reductase (*mvaA*) activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*.

Assays for measuring HMG-CoA reductase (*mvaA*) activity

MvaA is purified by standard methods using widely available molecular tags following expression at high level from *E. coli*. Enzymatic activity is monitored in the presence or absence of a putative inhibitory molecule of HMG-CoA reductase activity by following oxidation of NADPH to NADP spectrophotometrically at 340 nm. The assay is carried out in the following buffer: 0.25 mM NADPH, 0.25 mM HMG-CoA, 50 mM NaCl, 1 mM EDTA, 5 mM DTT, 25 mM KH_2PO_4 (pH 7.5). The assay is amenable to HTS in high density screening microtiter plates.

Forward reaction: Activity of HMG-CoA reductase (*mvaA*) from *Alloiococcus otitidis* in the presence or absence of a putative inhibitory molecule of HMG-CoA reductase activity is measured by reductive deacylation of HMG-CoA to mevalonate as measured the consumption of NADPH to NADP. Unlike other class II HMG Coa reductases, *MvaA* from *Alloiococcus otitidis*, like *S. aureus*, can use either NADPH or NADH cofactor in the reaction. The following kinetic data describe the reaction: $K_m(\text{HMG CoA}) = 40 \mu\text{M}$, $K_m(\text{NADPH}) = 70 \mu\text{M}$, $K_m(\text{NADP}) = 100 \mu\text{M}$ (12). This assay is inhibitable by the statin drug fluvastatin; the K_i was measured at 320 μM , which is four orders of magnitude higher than the K_i for class I HMG-CoA reductases.

Reverse reaction: The oxidative acylation of mevalonate to HMG-CoA in the presence or absence of a putative inhibitory molecule of HMG-CoA reductase activity is also monitored. The following kinetic data describes the reaction: $K_{m(\text{mevalonate})} = 670 \mu\text{M}$, $K_{m(\text{CoASH})} = 390 \mu\text{M}$, $K_{m(\text{NADP})} = 580 \mu\text{M}$ (12).

5

EXAMPLE 31

ALLOIOCOCCUS OTITIDIS ENCODED DIPHOSPHOMEVALONATE DECARBOXYLASE (MvAD)

Diphosphomevalonate decarboxylase, encoded by *mvaD*, the final enzyme
10 acting in the mevalonate pathway of IPP synthesis was cloned from *S. aureus* by
Wilding *et al* in 2000. Insertional inactivation of *mvaD* could only be accomplished
when the strains were supplemented with mevalonate, indicating that *mvaD* is
essential. The final step of the mevalonate pathway leading to IPP is the
decarboxylation and dehydration of mevalonate-5-pyrophosphate to form isopentenyl
15 diphosphate by MvaD (diphosphomevalonate decarboxylase).

MvaD homologues are well represented in gram-positive organisms (10).
Phylogenetic analysis revealed that the cluster of gram-positive enzymes (39-80%
identity) were well separated from the eukaryotic homologues, suggesting utility as
an antibacterial target. The *Alloiococcus otitidis* ORF- 1275b has been shown to
20 encode, by sequence homology, diphosphomevalonate decarboxylase (MvaD) (Seq.
ID No. 43). MvaD activity in the presence or absence of a putative inhibitory molecule
of diphosphomevalonate decarboxylase (MvaD) activity is used to identify novel
antimicrobial agents, which may be used to treat the disease(s) caused by
Alloiococcus otitidis. The protein encoded by the gene is set forth in Seq. ID No. 44.

25

Example 32

ALLOIOCOCCUS OTITIDIS ENCODED HMG CoA SYNTHASE (MVAS)

The second step of the mevalonate pathway leading to IPP is the irreversible
5 condensation of acetoacetyl-CoA and acetyl-CoA to form HMG-CoA by MvaS (HMG
CoA synthase). It has been shown that *mvaS* knockout mutant of *S. pneumoniae*
was attenuated for virulence. Due to its high specificity, essentiality, and importance,
mvaS is attractive as an antibacterial target. Homologue of this gene identified in
Alloiococcus otitidis is described in Example 5/Table 4 (Seq. ID No 35). The protein
10 encoded by the gene is set forth in Seq. ID No. 36.

HMG CoA SYNTHASE (MVAS) AS A TARGET FOR ANTI-INFECTIVE DEVELOPMENT

The *Alloiococcus otitidis* ORF- has been shown to encode, by sequence
15 homology, MvaS (HMG CoA synthase) (Seq. ID No. 35). MvaS activity in the
presence or absence of a putative inhibitory molecule of HMG-CoA synthase (*mvaS*)
activity is used to identify novel antimicrobial agents, which may be used to treat
disease caused by *Alloiococcus otitidis*.

Assays for measuring MvaS function

MvaS is purified by standard methods using widely available molecular tags
following expression at high level from *E. coli*. HMG-CoA synthase activity in the
presence or absence of a putative inhibitory molecule of HMG-CoA synthase (*mvaS*)
is assayed by measuring the loss of the enolate form of acetoacetyl-CoA
25 spectrophotometrically. The reaction is carried out in a buffer containing 50 mM Tris
(pH 9.75), 5.0 mM MgCl₂, 500 μM acetyl-CoA, 20 μM acetoacetyl-CoA and enzyme.
The enolate formed is monitored at 302 nm; therefore, as the acetoacetyl-CoA is
consumed the signal is depleted. Using this assay the following kinetic data is
measured: $K_{m(\text{acetyl-CoA})} = 350 \mu\text{M}$; $K_m^{\text{app}}(\text{acetoacetyl-CoA}) = 10 \mu\text{M}$. This assay is amenable
30 to HTS in high- high density screening microtiter plates.

Example 33**ALLOIOCOCCUS OTITIDIS ENCODED NICOTINAMIDE ADENINE DINUCLEOTIDE ADENYLYL
TRANSFERASE (NADD)**

5 Nicotinamide adenine dinucleotide (NAD) is an essential molecule in all living
cells. NAD is synthesized via a multi-step *de novo* pathway or via a pyridine salvage
pathway. The enzyme nicotinic acid mononucleotide adenylyl transferase (NaMN AT,
EC2.7.7.18) catalyzes the conversion of ATP and nicotinic acid mononucleotide
(NaMN) to nicotinic acid adenine dinucleotide (NaAD). The *nadD* gene, encoding
10 bacterial NaMN AT, is essential for NAD biosynthesis and bacterial cell survival.
NadD contains well-conserved the nucleotidyl transferase consensus sequence
(GXFXXHXGH). The adenylyl transferase encoded by the *nadD* gene prefers
NaMN over nicotinomide mononucleotide (NMN) as substrate. Due to its high
specificity, essentiality, and importance, *nadD* is attractive as an antibacterial target.
15 Homologue of this gene identified in *Alloiococcus otitidis* is described in Example
5/Table 4 (Seq. ID No 91). The protein encoded by the gene is set forth in Seq. ID
No. 92.

20 **NICOTINAMIDE ADENINE DINUCLEOTIDE ADENYLYL TRANSFERASE (NADD)
AS A TARGET FOR ANTI-INFECTIVE DEVELOPMENT**

The *Alloiococcus otitidis* ORF- has been shown to encode, by sequence
homology, nicotinomide adenine dinucleotide adenylyl transferase (NadD) (Seq. ID No.
91). NadD activity in the presence or absence of a putative inhibitory molecule of
25 NadD activity is used to identify novel antimicrobial agents, which may be used to
treat disease caused by *Alloiococcus otitidis*.

Assays for measuring NadD function**Discontinuous assay**

30 NadD activity in *Alloiococcus otitidis* is measured in the presence or
absence of a putative inhibitory molecule of NadD activity. NadD converts
nicotinic acid mononucleotide (NaMN) and adenosine triphosphate (ATP) to
nicotinic acid dinucleotide (NaAD) and pyrophosphate (PP_i). Each PP_i
molecule produced by the NadD reaction is then converted to two phosphate

(P_i) molecules in the presence of inorganic pyrophosphatase (PPase). The P_i molecules present are quantitated with a malachite green reagent at 660 nm.

HPLC-based assay: Enzyme activity is measured by HPLC quantitation of the reaction products. A neutralized aliquots from the reaction described above was injected into an HPLC system utilizing a 250 x4.6 mm Supelcosil LC-18 5µm reversed-phase column. The elution conditions: 9 min at 100% buffer A (0.1 M potassium phosphate buffer, pH6.0, 6 min at up to 12% buffer B (buffer a, containing 20% methanol, 2.5 min at up to 45% buffer B, 2.5 min at up to 100% buffer B, and hold at 100% buffer B for 5.5 min. The eluate absorbance was monitored at 254 nm.

Continuous assay

In bacteria, NadD combines nicotinic acid mononucleotide (NaMN) and adenosine triphosphate (ATP) to form nicotinic acid adenine dinucleotide (NaAD). NadE then converts NaAD into nicotinamide adenine dinucleotide (NAD) in the presence of ammonia and ATP. In the assay, the NAD product is reduced to NADH with alcohol dehydrogenase (ADH) and ethanol, thus permitting direct spectrometric detection of NADH at 340 nm wavelength. The coupled reaction above also includes inorganic pyrophosphatase (PPase) to prevent accumulation of the pyrophosphate byproduct from the consumption of ATP.

EXAMPLE 34

ALLOIOCOCCUS OTITIDIS ENCODED NICOTINAMIDE ADENINE DINUCLEOTIDE SYNTHASE (NADE)

NAD is a central compound in cellular metabolism. The final metabolic step in the pathway is conversion of nicotinamide adenine dinucleotide – product of NadD reaction – to NAD, a step catalyzed by the enzyme NAD synthetase (NadE). NaMN – substrate for NadD – can be formed by three different enzymatic reactions: in the *de novo* pathway from quinolinate, in Preiss-Handler salvage pathway from nicotinic acid, and in the nucleoside salvage pathway by deamination of nicotinamide mononucleotide. In bacteria, there are no known alternatives for the metabolic steps between NaMN and NAD. Mutants blocked in these steps cannot be recovered as auxotrophs since the required metabolites are not taken up by cells. In

the bacterial cells, the second substrate for NadE is ammonium, as opposed to glutamine for eukaryotes. NadE is an essential and conserved protein in the eubacterial nicotinamide adenine dinucleotide (NAD) biosynthesis pathway. Homologue of this gene identified in *Alloicoccus otitidis* is described in Example 5/ Table 4 (Seq. ID No 49). The protein encoded by the gene is set forth in Seq. ID No. 50.

Assays for measuring NadE function:

The *Alloicoccus otitidis* ORF- has been shown to encode, by sequence homology, nicotinamide adenine dinucleotide adenylyl synthase (NadE) (Seq. ID No. 49). NadE activity in the presence or absence of a putative inhibitory molecule of NadE activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloicoccus otitidis*.

DISCONTINUOUS ASSAY:

In assay, NadE converts nicotinic acid adenine dinucleotide (NaAD) into nicotinamide adenine dinucleotide (NAD) in the presence of ammonia and ATP. Each PP_i molecule produced by the NadE reaction can then be converted to two phosphate (P_i) molecules in the presence of inorganic pyrophosphatase (PPase). The P_i molecules present can then be quantitated with a malachite green reagent at 660 nm.

HPLC-based assay:

Enzyme activity can be measured by HPLC quantitation of the reaction products. A neutralized aliquots from the reaction described above was injected into an HPLC system utilizing a 250 x 4.6 mm Supelcosil LC-18 5μm reversed-phase column. The elution conditions: 9 min at 100% buffer A (0.1 M potassium phosphate buffer, pH 6.0), 6 min at up to 12% buffer B (buffer a, containing 20% methanol), 2.5 min at up to 45% buffer B, 2.5 min at up to 100% buffer B, and hold at 100% buffer B for 5.5 min. The eluate absorbance was monitored at 254 nm (1).

Continuous assay:

Coupled NadD-NadE assay. NadD and NadE can be detected in one continuous coupled assay. In first reaction, NadD combines nicotinic acid mononucleotide (NaMN) and adenosine triphosphate (ATP) to form nicotinic acid adenine dinucleotide (NaAD). NadE then converts NaAD into nicotinamide adenine dinucleotide (NAD) in the presence of ammonia and ATP. In the assay, the NAD product is reduced to NADH with alcohol dehydrogenase (ADH) and ethanol, thus permitting direct spectrometric detection of NADH at 340 nm wavelength. The coupled reaction above also includes inorganic pyrophosphatase (PPase) to prevent accumulation of the pyrophosphate byproduct from the consumption of ATP (this method can be use as HTS format).

NadE assay. In assay, NadE converts NaAD into nicotinamide adenine dinucleotide (NAD) in the presence of ammonia and ATP. The NAD product is reduced to NADH with alcohol dehydrogenase (ADH) and ethanol, thus permitting direct spectrometric detection of NADH at 340 nm wavelength. The reaction above also includes inorganic pyrophosphatase (PPase) to prevent accumulation of the pyrophosphate byproduct from the consumption of ATP (this method can be use as HTS format).

EXAMPLE 35**ALLOIOCOCCUS OTTIDIS ENCODED PUTATIVE MEMBRANE PROTEIN *NOR*A**

An efflux transporter NorA that was originally identified in *Staphylococcus aureus* belongs to the family of multidrug resistance (MDR) transporters. NorA is encoded by chromosomally-located *norA* gene, it has broad substrate specificity and mediates resistance to various lipophilic and monocationic compounds such as ethidium bromide (EtBr), cetrимide, benzalkonium chloride, rhodamine 6G, tetraphenylphosphonium (TPP), chloramphenicol as well as some hydrophilic quinolones such as norfloxacin, ciprofloxacin and oxafloxacin. Increased levels of *norA* expression are associated with single nucleotide changes upstream of *norA* in a putative promoter/operator region and lead to increased pleiotropic resistance. NorA is a putative membrane protein with 12 predicted membrane-spanning domains and is classified as a member of major facilitator superfamily (MFS), a subgroup of MDR

transporters characterized by the presence of 12-14 transmembrane segments and the use of proton motive force as an energy source for drug efflux. NorA homologs that belong to MFS family include Bmr and Blt of *Bacillus subtilis*, EmeA of *Enterococcus faecalis* and PmrA of *Streptococcus pneumonia*. The expression of *bmr* gene in *B. subtilis* is upregulated by the product of adjacent *bmR* gene in the presence of inducers (rhodamine 6G and TPP), and there is an evidence that expression of *norA* in *S. aureus* is regulated by AlrS-AlrR two-component regulatory system.

It remains unknown whether the efflux of various toxins is a primary function of NorA. When overexpressed in *E. coli*, *norA* produces resistance to a broad range of substrates including fluoroquinolones. Everted membrane vesicles prepared from *norA*-expressing *E. coli* exhibit energy-dependent transport of norfloxacin, the transfer is abolished by cyanide m-chlorophenylhydrazine (CCCP) and nigericin but not by valinomycin indicating that NorA-mediated transfer is coupled to the proton gradient of cell membrane. Norfloxacin uptake in everted vesicles as well as NorA-associated resistance phenotype is inhibited by reserpine and verapamil that also inhibit other MDR transporters and are toxic to mammalian cells. Histidine-tagged NorA (NorA-His) was recently overexpressed and purified from *E. coli*, reconstituted into both everted membrane vesicles and proteoliposomes and was shown to function as a self-sufficient efflux pump using fluorescent dye Hoechst 33342. Due to its high specificity, essentiality, and importance, *norA* is attractive as an antibacterial target. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 67). The protein encoded by the gene is set forth in Seq. ID No. 68.

NORA AS A TARGET FOR ANTI-INFECTIVE DEVELOPMENT

The *Alloiococcus otitidis* ORF- has been shown to encode, by sequence homology, NorA (Seq. ID No. 67). NorA activity in the presence or absence of a putative inhibitory molecule of NorA activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*. Because of broad substrate specificity of NorA, NorA inhibitors should be particularly useful against pathogens that possess multiple drug resistance.

Whole-cell high-throughput screen (HTS) assay that measures NorA activity in the presence or absence of a putative inhibitory molecule of *Alloiococcus otitidis*. NorA activity is used to identify potential inhibitors of NorA activity. The assay utilizes *B. subtilis* strain ($\Delta\Delta$ NA) that has both Bmr and Blt genetically inactivated while *Alloiococcus otitidis* NorA is supplied on the plasmid expression vector. The screen is based on the reversing of the resistance of $\Delta\Delta$ NA to EtBr. The exponentially growing cells are inoculated into the wells of a 96-well plate to $OD_{600}=0.001$, the compounds are added at 20 μ g/ml and EtBr is added at 10 μ g/ml. Plates are incubated for 18 hrs at 37°C and examined for growth. Compounds that inhibit growth are subsequently tested in the presence/absence of EtBr for toxicity and effectivity. The efflux of EtBr from cells is monitored as described previously. The exponentially growing cells are loaded with EtBr at a concentration of 10 μ g/ml for 20 min at 37°C in the presence of reserpine (20 μ g/ml). Cells are centrifuged, resuspended to an $OD_{600}=0.2$ in a minimal medium GM1 alone or in the presence of inhibitor compound. Fluorescence of EtBr is monitored on a fluorimeter at an excitation λ of 530 nm and emission λ of 600 nm..

MONITORING OF HOECHST 33342 EFFLUX

The efflux of fluorescent dye Hoechst 33342 from either everted membrane vesicles prepared from *Alloiococcus otitidis* His-NorA overexpressing *E. coli* or a proteoliposomes reconstituted with *Alloiococcus otitidis* His-NorA is also used to monitor NorA activity in the presence or absence of putative inhibitors of NorA. Everted membrane vesicles are diluted into 2 ml of 50 mM potassium HEPES (pH 7.2), 8.5 mM NaCl, 2 mM magnesium sulfate at a final protein concentration of 40 μ g/ml. NorA is activated by the addition of either 0.5 mM lactate or 0.1 mM Mg^{2+} -ATP. Hoechst 33342 is used in a range of 12.5 to 200 nM. Inhibitors are added at various concentrations prior to the addition of Hoechst 33342. Fluorescence change is monitored at excitation and emission wavelengths of 355 and 457 nm respectively in a FluoroMax spectrofluorimeter. For proteoliposome assay, the His-NorA proteoliposomes are diluted into a cuvette containing 2 ml of 20 mM potassium phosphate, 50 mM potassium sulfate, 2 mM magnesium sulfate (pH 7.0) at a protein concentration of 10 μ g/ml. The inhibitor compounds and Hoechst 33342 are added at various concentrations and the fluorescence is measured as described previously.

EXAMPLE 36

ALLOIOCOCCUS OTITIDIS ENCODED OBG GTPASE

5 The *obg* gene is the second gene in a two-gene operon along with the stage-
O sporulation gene *spoOB* in *B. subtilis*. SpoOB is central to the phospho-relay
signal cascade that initiates sporulation. Obg is a member of the GTPase
superfamily by virtue of homology throughout a small portion of the protein that in
other members of the family is responsible for nucleotide (GTP/GDP) binding. Obg
10 is essential for growth. Initiation of sporulation is thought to be triggered by changes
in the GTP content of the cell; therefore, the presence of a GTP binding protein in an
operon with a central player in the process is suggestive of a role for Obg in sensing
GTP levels and transmitting a signal to SpoOB.

It has been shown that Obg is involved in activation of the σ^B transcription
15 factor in *B. subtilis* in response to environmental stress. Cells were depleted of Obg
utilizing a construct that put *obg* under the control of an inducible (P_{lac}) promoter.
Depletion of IPTG resulted in bacteria that failed to activate σ^B . These studies further
showed by yeast-two-hybrid analysis that Obg interacted with several known σ^B
regulators, the so-called Rsb proteins.

20 The role Obg plays in transmitting signals important for sporulation and
activation of the stress sigma factor may be indicative of the activities that small GTP
binding proteins carry out in triggering cell division in response to GTP levels. Due to
its high specificity, essentiality, and importance, *obg* is attractive as an antibacterial
target. Homologue of this gene identified in *Alloiococcus otitidis* is described in
25 Example 5/Table 4 (Seq. ID No 71). The protein encoded by the gene is set forth in
Seq. ID No. 72.

OBG AS A TARGET FOR ANTI-INFECTIVE DEVELOPMENT

30 Obg is essential for bacterial viability. Conditional lethal alleles revealed that
Obg is required for early events in sporulation and is involved in transmitting signals
require for activation of the stress sigma factor. The *Alloiococcus otitidis* ORF- has
been shown to encode, by sequence homology, *obg* (Seq. ID No.71). Obg activity in
the presence or absence of a putative inhibitory molecule of Obg activity is used to

identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*.

Nucleotide binding

5 Obg binding to nucleotide in the presence or absence of putative antimicrobials, which inhibit Obg activity, is monitored by a simple filter-binding assay. *Alloiococcus otitidis* Obg (1-5 µg) is incubated with $\alpha^{32}\text{P}$ -GTP (0.2 µCi) in a buffer consisting of 50 mM Tris (pH 8.5), 1.5 mM MgCl_2 , 0.1 mM EDTA, 200 mM KCl, 10% glycerol for 30 minutes to 3 hours at 37°C. A portion of the reaction mix is
10 spotted on nitrocellulose membrane, washed (50 mM Tris (pH 8.5), 1.5 mM MgCl_2 , 1 mM DTT) and dried. The membrane is then exposed to X-ray film. Alternatively, the spots are excised and counted. This assay is directly amenable to HTS using filter plates.

GTPase activity

15 The GTP hydrolytic activity of Obg is monitored using thin-layer chromatography (1, 2, 10). Obg and $\alpha^{32}\text{P}$ -GTP are incubated in 50 mM Tris (pH 8.5), 1.55 mM MgCl_2 , 0.1 mM EDTA, 200 mM KCl, 10% glycerol for 30 minutes at 37°C. An aliquot of the reaction is placed on PEI cellulose and the strip developed with 0.5
20 M KH_2PO_4 , 1.0 M NaCl (pH 3.7). The spots conforming to GDP and GTP are identified by UV shadowing, excised and counted.

 Alternatively, the hydrolysis of $\gamma^{32}\text{P}$ -GTP is monitored by assaying for liberated P_i (12). Obg and $\alpha^{32}\text{P}$ -GTP are incubated in 50 mM Tris (pH 8.5), 1.5 mM MgCl_2 , 0.1 mM EDTA, 100 mM KCl, 10% glycerol for 30 minutes to 3 hours at 37°C.
25 The reaction is stopped by the addition of a slurry of charcoal in 1 mM Kpi (pH 7.5), which selectively binds the GTP and GDP. The liberated P_i in the supernatant is monitored by Cerenkov counting. Free P_i is also monitored with the Malachite Green reagent.

Autophosphorylation

30 Obg autophosphorylation is monitored by incubating Obg with $\gamma^{32}\text{P}$ -GTP in 50 mM Tris (pH 8.5), 1.5 mM MgCl_2 , 0.1 mM EDTA, 100 mM KCl, 10% glycerol for 30

minutes at 37°C. Samples are analyzed following separation on SDS polyacrylamide gels, drying the gel and exposure to film.

EXAMPLE 37.

5 **RPOA, RPOB, RPOC, AND RPOD, THE GENES ENCODING THE SUBUNITS COMPRISING**
 ALLOIOCOCCUS OTTIDIS RNA POLYMERASE: ALPHA, BETA, BETA', AND SIGMA.

RNA polymerase is an enzyme comprised of multiple highly conserved subunits which catalyzes the DNA template directed polymerization of ribonucleic
10 nucleotides into ribonucleic acid. It is composed of a core enzyme, $\alpha_2\beta\beta'$, along with a fifth subunit present in stoichiometric amounts, ω which can catalyze RNA synthesis non-specifically. Holoenzyme is formed by the introduction of the subunit σ , which enhances gene promoter recognition and allows specificity. Homologs of the genes identified in *Alloiococcus otitidis* are described in Example 5/Table 4 (Seq.
15 ID Nos 7, 9, 11, and 13). The amino acid sequence of the protein encoded by these genes are set forth in Seq. ID Nos. 8, 10, 12 and 14.

Functions for the individual subunits have been defined biochemically, and interactions between them have now been deduced structurally by crystallographic analysis of the enzyme from *Thermatoga thermophila*, and to a lesser extent,
20 *Escherichia coli*. The alpha subunit, encoded by *rpoA*, is required for enzyme assembly. It also interacts with transcription factors and with DNA elements involved in enhanced promoter strength. Beta, encoded by *rpoB*, is involved in initiation and elongation of the polymerization product. Beta' (encoded by *rpoC*), is responsible for binding of the enzyme to the DNA template. Omega is required to restore denatured
25 RNA polymerase to function *in vitro*. Finally, sigma, encoded by *rpoD*, directs the enzyme to promoters on the template to enhance specificity of transcription (polymerization).

ALLOIOCOCCUS OTITIDIS RNA POLYMERASE: ALPHA, BETA, BETA', AND SIGMA AS A TARGET FOR ANTI-INFECTIVE DEVELOPMENT

Bacterial RNA polymerase is a validated target for antimicrobial chemotherapy in that several inhibitors have been identified and at least one, rifampin, is in use clinically. *Alloiococcus otitidis* RNA polymerase holoenzyme is essential for bacterial viability. The *Alloiococcus otitidis* ORFs- have been shown to encode, by sequence homology, RNA polymerase holoenzyme (Seq. ID Nos. 7, 9, 11 and 13). *Alloiococcus otitidis* RNA Polymerase activity in the presence or absence of a putative inhibitory molecule of *Alloiococcus otitidis* RNA Polymerase activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*.

Assays for the activity of RNA polymerase

Genes encoding the subunits of *Alloiococcus otitidis* RNA polymerase can be obtained using polymerase chain reaction amplification of the genomic region encoding them. The genes are subcloned into a standard expression vector either containing an amino acid tag for ease of purification or not. The enzyme are overexpressed in *Escherichia coli* and purified using a standard tag system or conventional chromatography .

Because RNA polymerase catalyzes the incorporation of single ribonucleotides into RNA, the incorporation of radiolabelled nucleotides into larger oligonucleotides is monitored to measure activity of the enzyme in the presence or absence of putative inhibitors of RNA polymerase activity. An automated high throughput filtration assay has been previously described for *E. coli* polymerase which uses filterplates containing a hydrophobic membrane and DEAE beads to capture polymerized RNA. G-less supercoiled DNA is used as a template at 6 ug/ml. Reaction contained 0.5 mM ATP, 0.1 mM UTP, 0.3 mM CTP, approximately 100,000 counts per minute (per 100 ul) [γ -³³P] CTP (2000 Ci/mmol, NEN/DuPont), 4 % polyethylene glycol, 4 mM DTT, 10 mM MgCl₂, in 50 mM Tris-acetate (pH 7.8), and 100 mM potassium acetate. The reaction is carried out at 34 degrees C for 40 minutes, with 10% DMSO present in all reactions. The reaction was stopped by adding 100 ul 15% DEAE-Sepacel bead slurry in 50% methanol, 20 mM EDTA, and 0.02% NP-40. The reaction was incubated for 40-60 minutes at room temperature without shaking, and then transferred to a unifilter plate on a filtermate cell harvester. The wells were washed

six times with 2X PBS and 0.1% NP-40. After washing the bottom of the plate was sealed, and 50 ul scintillation counting liquid was added. Radioactivity was counted using a microplate scintillation counter.

Deconvolution assays are carried out by measuring the inhibition of sigma activity. Because sigma is required only for promoter specificity, polymerization may occur non-specifically if sigma is inhibited. Consequently a second assay is described above that is used to deconvolute activity against sigma.

The binding of putative inhibitory compounds to core enzyme. Several techniques are utilized to determine the interaction of inhibitors with individual subunits and include nuclear magnetic resonance and capillary electrophoresis.

EXAMPLE 38

YPHC, ENCODING A SMALL GTPASE OF UNKNOWN FUNCTION FROM *ALLOIOCOCCUS OTITIDIS*

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The *yphC* was initially identified in *Bacillus subtilis* in a collaboration between Wyeth and Millennium pharmaceuticals as being essential for growth by insertional mutagenesis. Subsequently it was determined that YphC, the encoded protein, contained two GTPase domains and had some homology to *era*. It was further identified in *Thermatoga maritima* and *Escherichia coli*. While no function has yet been determined for *yphC*, it appears that the carboxy terminal may contain an RNA binding site. In addition, site directed mutagenesis of four amino acids in the carboxy region were found to be lethal (unpublished results, Millennium). Under non-permissive conditions, strains carrying temperature sensitive alleles of the gene in *E. coli* become elongated, and chromosome segregation becomes aberrant, suggesting a role in cell division. Homologue of this gene identified in *Alloiococcus otitidis* is described in Example 5/Table 4 (Seq. ID No 73). The protein encoded by the gene is set forth in Seq. ID No. 74.

YphC from *Alloiococcus otitidis* as a target for antimicrobial chemotherapy

YphC is an essential protein in *Bacillus subtilis* and *E. coli*, and is conserved among bacteria including *Alloiococcus otitidis*. The *Alloiococcus otitidis* ORF- has
5 been shown to encode, by sequence homology, YphC (Seq. ID No. 73). YphC activity in the presence or absence of a putative inhibitory molecule of YphC activity is used to identify novel antimicrobial agents, which may be used to treat disease caused by *Alloiococcus otitidis*. Consequently it is proposed here that an assay
10 which identified inhibitors of YphC from *Alloiococcus* would result in small molecules which can be developed into effect antimicrobial agents. Additionally, because of the conservation of the enzyme among bacteria, inhibitors of the protein's function from this organism should have broad spectrum activity.

Assays for the GTP hydrolysis by YphC

15 The YphC gene from *Alloiococcus otitidis* is obtained using polymerase chain reaction amplification of the genomic region encoding it. The gene is subcloned into a standard expression vector either containing an amino acid tag for ease of purification or not. The enzyme is then overexpressed in *Escherichia coli* and purified using a standard tag system or conventional chromatography. Activity of
20 YphC in the presence or absence putative antimicrobial agents is monitored using the assay system described below.

GTP hydrolysis – detection by thin layer chromatography: Reaction is carried out in a 50 ul reaction of 50 mM Tris-Cl (pH 7.5), 400 mM KCl, 5 mM MgCl₂,
25 1 mM DTT, 10 uM [α -³²P] GTP, and 10 ug purified YphC, at 37 degrees for 10 minutes. The reaction is terminated by transfer of 5 ul samples to 10 ul of ice-cold 20 mM EDTA. Portions are spotted onto polyethyleneimine-cellulose thin layer chromatography plates, which are developed in 0.75 KH₂PO₄ (pH 3.65). The plate is autoradiographed to identify hydrolysis products.

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WHAT IS CLAIMED IS:

- 5 1. A purified or isolated *Alloiococcus otitidis* nucleic acid sequence comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, wherein expression of said nucleic acid is essential for the proliferation of a cell.
- 10 2. A purified or isolated nucleic acid of *Alloiococcus otitidis* comprising a fragment of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105 said fragment selected from the group consisting of fragments comprising at least 10, at least 20, at least 25, at least 30, at least 50 and more than 50 consecutive nucleotides of one of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.
- 15
3. A purified or isolated antisense nucleic acid comprising a nucleotide sequence complementary to at least a portion of an intragenic sequence, intergenic sequence, sequences spanning at least a portion of two or more genes, 5' noncoding region, or 3' noncoding region within an operon comprising a proliferation-required gene of *Alloiococcus otitidis* whose activity or expression is inhibited by an antisense nucleic acid and selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.
- 20
- 25 4. A purified or isolated nucleic acid comprising a nucleotide sequence having at least 70% identity to a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, fragments comprising at least 25 consecutive nucleotides selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, the nucleotide sequences complementary to one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, and the sequences complementary to fragments comprising at least 25 consecutive nucleotides
- 30

of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.

5. A vector comprising a promoter operably linked to a nucleic acid encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of any one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.
5. A purified or isolated polypeptide of *Alloiococcus otitidis* comprising a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or a fragment selected from the group consisting of fragments comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of one of the said polypeptides.
6. A purified or isolated *Alloiococcus otitidis* polypeptide comprising a amino acid sequence having at least 25% amino acid identity to a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or at least 25% amino acid identity to a fragment comprising at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.
7. A purified or isolated *Alloiococcus otitidis* polypeptide comprising selected from one of the even numbered sequences set forth in Seq. ID Nos: 2 to Seq. ID Nos: 106, wherein the polypeptide is essential for the proliferation of a cell.

8. A method of producing an *Alloiococcus otitidis* polypeptide comprising introducing into a cell a vector comprising a promoter operably linked to a nucleic acid comprising a nucleotide sequence encoding a polypeptide whose expression is essential for the proliferation and viability of *Alloiococcus otitidis*, and which is inhibited by an antisense nucleic acid, and which is selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105.
9. A method of inhibiting the proliferation of *Alloiococcus otitidis* in an individual comprising inhibiting the activity or reducing the amount of a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105 or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product.
10. A method for identifying a compound which influences the activity of an *Alloiococcus otitidis* gene product, which is required for proliferation, said gene product comprising a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, said method comprising:
- (a) contacting said gene product with a candidate compound; and
 - (b) determining whether said compound influences the activity of said gene product.
11. A method for identifying a compound or an antisense nucleic acid having the ability to reduce activity or level of a *Alloiococcus otitidis* gene product, which is required for proliferation, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid

comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, said method comprising the steps of:

- 5 (a) contacting a target gene or RNA encoding said gene product with a candidate compound or antisense nucleic acid; and
(b) measuring the activity of said target.

10 13. A method for inhibiting cellular proliferation of *Alloiococcus otitidis* comprising introducing an effective amount of a compound with activity against a gene whose activity or expression is essential for cellular proliferation, and which is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or a compound with activity against the product of said gene into a population of *Alloiococcus otitidis* cells expressing said gene.

15

13. A composition comprising an effective concentration of an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, or a proliferation-inhibiting portion thereof in a pharmaceutically acceptable carrier.

20

14. A method for identifying a compound having the ability to inhibit proliferation of *Alloiococcus otitidis* cell comprising:

- 25 (a) identifying a homologue of a gene or gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, in a test cell, wherein said test cell is not *Alloiococcus otitidis*;
(a) identifying an inhibitory nucleic acid sequence which inhibits the
30 activity of said homologue in said test cell;
(b) contacting said test cell with a sublethal level of said inhibitory nucleic acid, thus sensitizing said cell;
(c) contacting the sensitized cell of step (c) with a compound; and

(d) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said inhibitory nucleic acid.

5 16. A method for identifying a compound having activity against a biological pathway required for proliferation comprising:

10 (a) sensitizing a cell by providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding a gene product required for proliferation, wherein the activity or expression of said gene product is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, in said cell to reduce the activity or amount of said gene product;

15 (a) contacting the sensitized cell with a compound; and

(b) determining the degree to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

20 17. A method for identifying a compound having the ability to inhibit one of the *Alloiococcus otitidis* polypeptides encoded by a polynucleotide selected from one of odd numbered sequences set forth in Seq. ID Nos: 1 to Seq. ID Nos: 105, and which is essential for cellular proliferation comprising:

25 (a) contacting a cell which expresses the polypeptide with the compound; and

(b) determining whether said compound reduces proliferation of said contacted cell by acting on said gene product.

30 18. A method for identifying a compound having the ability to inhibit one of the purified and isolated *Alloiococcus otitidis* polypeptides selected from one of the even numbered sequences set forth in Seq. ID No.: 2 to Seq. ID No.: 106, and which is essential for cellular proliferation comprising:

- (c) contacting the purified and isolated polypeptide with the compound *in vitro* in the presence or absence of a substrate, which is essential for the activity of the polypeptide; and
- (d) determining the effect of the compound on the polypeptide by measuring the effect of the polypeptide on the substrate.

19. A compound which interacts with an *Alloiococcus otitidis* polypeptide selected from one of the even numbered sequences set forth in Seq. ID No.: 2 to Seq. ID No.: 106 and inhibits its activity.
20. A method for manufacturing an antimicrobial compound comprising the steps of screening one or more candidate compounds to identify a compound that reduces the activity or level of an *Alloiococcus otitidis* polypeptide selected from one of the even numbered sequences set forth in Seq. ID No.: 2 to Seq. ID No.: 106, said polypeptide comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105; and manufacturing the compound so identified.
21. A compound which inhibits proliferation of *Alloiococcus otitidis* by interacting with a gene encoding a polypeptide that is required for proliferation or with a polypeptide required for proliferation, wherein said polypeptide is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105, polypeptide encoded by a nucleic acid having at least 70% nucleotide sequence identity to a nucleic acid encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105, a polypeptide having at least 25% amino acid identity to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected one of the odd numbered sequences set forth in Seq. ID No.: 1 to

Seq. ID No. 105, a polypeptide encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105 under stringent conditions, a gene product encoded by a nucleic acid
5 comprising a nucleotide sequence which hybridizes to a nucleic acid selected from one of the odd numbered sequences set forth in Seq. ID No.: 1 to Seq. ID No. 105 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from one of the odd numbered sequences set forth in
10 Seq. ID No.: 1 to Seq. ID No. 105.

SEQUENCE LISTING

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Glu Ile Gly Ile Asp Leu Asp Ile Gly Glu Asp Ser Ile Arg Val Lys
275 280 285

Ala Pro Ser Lys Pro Leu Gln Pro Val Thr Ile Lys Thr Leu Pro Tyr
290 295 300

Pro Gly Phe Ala Thr Asp Leu Gln Gln Pro Ile Thr Pro Leu Leu Leu
305 310 315 320

Leu Ala Lys Gly Glu Ser Val Ile Thr Asp Thr Ile Tyr Pro Lys Arg
325 330 335

Val Lys His Ile Pro Glu Leu Glu Arg Met Gly Ala Asn Ile Arg Val
340 345 350

Glu Ser Asp Ile Ile Leu Ile Glu Gly Gly His Pro Leu Lys Gly Ala
355 360 365

Glu Val Glu Ala Ser Asp Leu Arg Ala Gly Ala Cys Leu Ile Asn Ala
370 375 380

Gly Leu Ile Ala Glu Gly Gln Thr Glu Ile Thr Gly Val Asp Lys Ile
385 390 395 400

Leu Arg Gly Tyr Ser His Ile Val Glu Lys Leu Asn Asp Leu Gly Ala
405 410 415

Asp Val Tyr Met Gln Glu Gly Glu Asp
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<211> 612

<212> DNA

<213> *Alloiococcus otitidis*

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Met	His	Arg	Gln	Asp	Leu	Asn	Arg	Glu	Arg	Lys	Ser	Asp	Val	Glu		
1				5				10						15		

tta	aaa	gag	ttt	gat	gga	aag	aaa	aaa	gaa	gaa	cta	gcc	atg	att	gat	96
Leu	Lys	Glu	Phe	Asp	Gly	Lys	Lys	Lys	Glu	Glu	Leu	Ala	Met	Ile	Asp	
				20					25					30		

gtg	gcc	aag	gcc	att	tta	gac	cag	gtc	cat	gac	ttg	atg	cac	ttc	aac	144
Val	Ala	Lys	Ala	Ile	Leu	Asp	Gln	Val	His	Asp	Leu	Met	His	Phe	Asn	
			35				40						45			

gac	ctc	ttg	agt	gaa	gtg	tct	gaa	tat	cta	gac	ttg	tca	gat	gac	gag	192
Asp	Leu	Leu	Ser	Glu	Val	Ser	Glu	Tyr	Leu	Asp	Leu	Ser	Asp	Asp	Glu	
		50					55					60				

atc	gaa	agc	ggt	atg	ggc	caa	ttt	tac	acc	gat	tta	aat	att	gac	ggt	240
Ile	Glu	Ser	Gly	Met	Gly	Gln	Phe	Tyr	Thr	Asp	Leu	Asn	Ile	Asp	Gly	
	65					70					75					

cgc	ttc	atc	tct	tta	ggc	gac	aac	cat	tgg	ggc	tta	cgt	gaa	tgg	tat	288
Arg	Phe	Ile	Ser	Leu	Gly	Asp	Asn	His	Trp	Gly	Leu	Arg	Glu	Trp	Tyr	
80					85					90					95	

cca	gtc	gat	tct	atc	gat	gaa	gag	ttg	acc	cac	gac	aat	gac	ctg	gag	336
Pro	Val	Asp	Ser	Ile	Asp	Glu	Glu	Leu	Thr	His	Asp	Asn	Asp	Leu	Glu	
				100					105					110		

aag	gtc	aca	ccc	aag	cag	gcg	gaa	gac	ggc	ttt	gat	gac	tta	gag	cat	384
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Lys Val Thr Pro Lys Gln Ala Glu Asp Gly Phe Asp Asp Leu Glu His
 115 120 125
 gtc gaa aaa gaa gtg atg gat gac gca aaa gaa gaa tta gat gac cag 432
 Val Glu Lys Glu Val Met Asp Asp Ala Lys Glu Glu Leu Asp Asp Gln
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 gcc gtc aat gaa gat gaa gaa aat gtt gct cca gat gaa atc acc gac 480
 Ala Val Asn Glu Asp Glu Glu Asn Val Ala Pro Asp Glu Ile Thr Asp
 145 150 155
 gat gga gat gaa gac aag ctg gat gaa tac tct agc gat atc gaa gac 528
 Asp Gly Asp Glu Asp Lys Leu Asp Glu Tyr Ser Ser Asp Ile Glu Asp
 160 165 170 175
 ctc gaa gat gat cgt aag gct agc caa gac aag ctg tcc att gtt gac 576
 Leu Glu Asp Asp Arg Lys Ala Ser Gln Asp Lys Leu Ser Ile Val Asp
 180 185 190
 gac gaa gat gtc tta aca aat gat gac gat gag taa 612
 Asp Glu Asp Val Leu Thr Asn Asp Asp Asp Glu
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<213> *Alloiococcus otitidis*

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 20 25 30

Ala Lys Ala Ile Leu Asp Gln Val His Asp Leu Met His Phe Asn Asp
 35 40 45

Leu Leu Ser Glu Val Ser Glu Tyr Leu Asp Leu Ser Asp Asp Glu Ile
 50 55 60

Glu Ser Gly Met Gly Gln Phe Tyr Thr Asp Leu Asn Ile Asp Gly Arg
 65 70 75 80

Phe Ile Ser Leu Gly Asp Asn His Trp Gly Leu Arg Glu Trp Tyr Pro
 85 90 95

Val Asp Ser Ile Asp Glu Glu Leu Thr His Asp Asn Asp Leu Glu Lys
 100 105 110

Val Thr Pro Lys Gln Ala Glu Asp Gly Phe Asp Asp Leu Glu His Val
115 120 125

Glu Lys Glu Val Met Asp Asp Ala Lys Glu Glu Leu Asp Asp Gln Ala
130 135 140

Val Asn Glu Asp Glu Glu Asn Val Ala Pro Asp Glu Ile Thr Asp Asp
145 150 155 160

Gly Asp Glu Asp Lys Leu Asp Glu Tyr Ser Ser Asp Ile Glu Asp Leu
165 170 175

Glu Asp Asp Arg Lys Ala Ser Gln Asp Lys Leu Ser Ile Val Asp Asp
180 185 190

Glu Asp Val Leu Thr Asn Asp Asp Asp Glu
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<210> 9

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gat ggc aaa ttc ggt aag ttt gtt gtt gaa cca ttg gaa cgt ggt tat 96
Asp Gly Lys Phe Gly Lys Phe Val Val Glu Pro Leu Glu Arg Gly Tyr
20 25 30

ggg act acc tta ggg aat tcc tta cgc cgc atc tta tta tca tca cta 144
Gly Thr Thr Leu Gly Asn Ser Leu Arg Arg Ile Leu Leu Ser Ser Leu
35 40 45

ccg ggt gct gcg gtc acc aat att caa att gat ggt gtt ttg cat gag 192
Pro Gly Ala Ala Val Thr Asn Ile Gln Ile Asp Gly Val Leu His Glu
50 55 60

ttt aca gct att gat ggt gtg gtt gaa gat gtg act tcc atc atc tta 240
Phe Thr Ala Ile Asp Gly Val Val Glu Asp Val Thr Ser Ile Ile Leu
65 70 75 80

aac ctg aaa aaa ctg gct tta aaa ctt cat act gaa gaa aca aaa aca 288

Asn	Leu	Lys	Lys	Leu	Ala	Leu	Lys	Leu	His	Thr	Glu	Glu	Thr	Lys	Thr	
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att	gaa	ttg	gat	att	gaa	ggc	cct	gct	gaa	gtg	aca	gca	gct	gat	att	336
Ile	Glu	Leu	Asp	Ile	Glu	Gly	Pro	Ala	Glu	Val	Thr	Ala	Ala	Asp	Ile	
			100					105					110			
att	act	gat	agt	gat	gtt	gag	att	atg	aat	cca	gac	cta	tac	ttg	tgt	384
Ile	Thr	Asp	Ser	Asp	Val	Glu	Ile	Met	Asn	Pro	Asp	Leu	Tyr	Leu	Cys	
		115					120					125				
act	gtt	tct	gaa	ggg	ggg	cat	tta	cac	atc	cgg	atg	gaa	gca	gaa	act	432
Thr	Val	Ser	Glu	Gly	Gly	His	Leu	His	Ile	Arg	Met	Glu	Ala	Glu	Thr	
	130					135					140					
ggg	aga	ggg	tat	gtg	aat	gca	gag	cac	aac	aag	cat	gat	gat	atg	cca	480
Gly	Arg	Gly	Tyr	Val	Asn	Ala	Glu	His	Asn	Lys	His	Asp	Asp	Met	Pro	
145					150					155					160	
atc	ggg	gtt	ttg	cca	att	gat	tca	att	tat	acc	cca	att	agc	cgt	gtc	528
Ile	Gly	Val	Leu	Pro	Ile	Asp	Ser	Ile	Tyr	Thr	Pro	Ile	Ser	Arg	Val	
				165					170					175		
aac	tat	act	gtt	gaa	gac	acc	cgc	gtt	ggg	gaa	cgc	gag	caa	tat	gat	576
Asn	Tyr	Thr	Val	Glu	Asp	Thr	Arg	Val	Gly	Glu	Arg	Glu	Gln	Tyr	Asp	
			180					185					190			
aag	tta	acc	ctg	gat	att	tgg	aca	gat	gga	tcc	atc	tcc	cca	gag	gat	624
Lys	Leu	Thr	Leu	Asp	Ile	Trp	Thr	Asp	Gly	Ser	Ile	Ser	Pro	Glu	Asp	
		195				200						205				
ggc	ttg	agt	cta	gcg	gct	aag	atc	atg	aat	gaa	cac	ttg	aac	atc	ttc	672
Gly	Leu	Ser	Leu	Ala	Ala	Lys	Ile	Met	Asn	Glu	His	Leu	Asn	Ile	Phe	
	210					215					220					
atc	aac	tta	act	gag	caa	gca	cgt	gaa	gcg	gac	att	atg	gtt	gaa	aaa	720
Ile	Asn	Leu	Thr	Glu	Gln	Ala	Arg	Glu	Ala	Asp	Ile	Met	Val	Glu	Lys	
225					230					235					240	
gaa	gaa	gac	cag	aaa	gaa	aaa	atg	ctt	gag	atg	acc	atc	gaa	gag	ctt	768
Glu	Glu	Asp	Gln	Lys	Glu	Lys	Met	Leu	Glu	Met	Thr	Ile	Glu	Glu	Leu	
				245					250					255		
gat	tta	tct	gtt	cgg	tct	tac	aac	tgt	ttg	aaa	cgt	gct	ggc	atc	aat	816
Asp	Leu	Ser	Val	Arg	Ser	Tyr	Asn	Cys	Leu	Lys	Arg	Ala	Gly	Ile	Asn	
			260					265					270			
act	gtc	caa	gaa	cta	acg	gac	aaa	act	gaa	ccg	gaa	atg	atg	aaa	gtt	864
Thr	Val	Gln	Glu	Leu	Thr	Asp	Lys	Thr	Glu	Pro	Glu	Met	Met	Lys	Val	
		275					280					285				
cgc	aat	ctc	gga	cgt	aag	tca	tta	gaa	gaa	gtt	aaa	aac	aag	ctt	gat	912
Arg	Asn	Leu	Gly	Arg	Lys	Ser	Leu	Glu	Glu	Val	Lys	Asn	Lys	Leu	Asp	
	290					295					300					
gac	tta	gac	cta	agc	ttg	aaa	gaa	gaa	tag							942
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305

310

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<212> PRT

<213> Alloiococcus otitidis

<400> 10

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20 25 30

Gly Thr Thr Leu Gly Asn Ser Leu Arg Arg Ile Leu Leu Ser Ser Leu
35 40 45

Pro Gly Ala Ala Val Thr Asn Ile Gln Ile Asp Gly Val Leu His Glu
50 55 60

Phe Thr Ala Ile Asp Gly Val Val Glu Asp Val Thr Ser Ile Ile Leu
65 70 75 80

Asn Leu Lys Lys Leu Ala Leu Lys Leu His Thr Glu Glu Thr Lys Thr
85 90 95

Ile Glu Leu Asp Ile Glu Gly Pro Ala Glu Val Thr Ala Ala Asp Ile
100 105 110

Ile Thr Asp Ser Asp Val Glu Ile Met Asn Pro Asp Leu Tyr Leu Cys
115 120 125

Thr Val Ser Glu Gly Gly His Leu His Ile Arg Met Glu Ala Glu Thr
130 135 140

Gly Arg Gly Tyr Val Asn Ala Glu His Asn Lys His Asp Asp Met Pro
145 150 155 160

Ile Gly Val Leu Pro Ile Asp Ser Ile Tyr Thr Pro Ile Ser Arg Val
165 170 175

Asn Tyr Thr Val Glu Asp Thr Arg Val Gly Glu Arg Glu Gln Tyr Asp
180 185 190

Lys Leu Thr Leu Asp Ile Trp Thr Asp Gly Ser Ile Ser Pro Glu Asp
 195 200 205

Gly Leu Ser Leu Ala Ala Lys Ile Met Asn Glu His Leu Asn Ile Phe
 210 215 220

Ile Asn Leu Thr Glu Gln Ala Arg Glu Ala Asp Ile Met Val Glu Lys
 225 230 235 240

Glu Glu Asp Gln Lys Glu Lys Met Leu Glu Met Thr Ile Glu Glu Leu
 245 250 255

Asp Leu Ser Val Arg Ser Tyr Asn Cys Leu Lys Arg Ala Gly Ile Asn
 260 265 270

Thr Val Gln Glu Leu Thr Asp Lys Thr Glu Pro Glu Met Met Lys Val
 275 280 285

Arg Asn Leu Gly Arg Lys Ser Leu Glu Glu Val Lys Asn Lys Leu Asp
 290 295 300

Asp Leu Asp Leu Ser Leu Lys Glu Glu
 305 310

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 caa att gga ctg gct tca cca gag aaa atc cgt tca tgg tct cat ggt 99
 Gln Ile Gly Leu Ala Ser Pro Glu Lys Ile Arg Ser Trp Ser His Gly
 15 20 25
 gaa gtg aag aaa cct gaa acc att aac tac cgg aca tta aaa cct gaa 147
 Glu Val Lys Lys Pro Glu Thr Ile Asn Tyr Arg Thr Leu Lys Pro Glu
 30 35 40
 aaa gac ggt ttg ttc tgc gaa cgc att ttt ggc cca acc aag gac tat 195
 Lys Asp Gly Leu Phe Cys Glu Arg Ile Phe Gly Pro Thr Lys Asp Tyr

45	50	55	
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tgt gac cgt tgc ggt gtt gaa gtc acc aag tcg agt gtc aga cga gaa Cys Asp Arg Cys Gly Val Glu Val Thr Lys Ser Ser Val Arg Arg Glu 75 80 85 90			291
cgc atg ggc cac ttg gaa tta gca gct cct gtc acc cac att tgg tac Arg Met Gly His Leu Glu Leu Ala Ala Pro Val Thr His Ile Trp Tyr 95 100 105			339
ttc aag ggt att cca agt cgg atg ggc ctt atc tta gat atg agc cca Phe Lys Gly Ile Pro Ser Arg Met Gly Leu Ile Leu Asp Met Ser Pro 110 115 120			387
aga tcc ttg gaa gaa att atc tat ttt gcc tct tat gtt gtt att gac Arg Ser Leu Glu Glu Ile Ile Tyr Phe Ala Ser Tyr Val Val Ile Asp 125 130 135			435
ggt ggg gat acc ccg ctt gaa cgc aaa cag ctc tta act gaa cgt gaa Gly Gly Asp Thr Pro Leu Glu Arg Lys Gln Leu Leu Thr Glu Arg Glu 140 145 150			483
tac cgg gaa aac aaa agc aag tac ggc aat gaa ttc caa gct gaa att Tyr Arg Glu Asn Lys Ser Lys Tyr Gly Asn Glu Phe Gln Ala Glu Ile 155 160 165 170			531
gga gct gaa gct gtt cgg acc ttg cta aaa aat gtc gat ttg gaa caa Gly Ala Glu Ala Val Arg Thr Leu Leu Lys Asn Val Asp Leu Glu Gln 175 180 185			579
gaa gtt gct gac ctc aaa gaa atc tta gaa act gca act ggc caa aaa Glu Val Ala Asp Leu Lys Glu Ile Leu Glu Thr Ala Thr Gly Gln Lys 190 195 200			627
cgg acc cgg gct att cgt cgt tta gac att att gac tcc ttc aag tct Arg Thr Arg Ala Ile Arg Arg Leu Asp Ile Ile Asp Ser Phe Lys Ser 205 210 215			675
tcc aac aac aaa ccg gaa tgg atg gtc ttg gat gct att cca att atc Ser Asn Asn Lys Pro Glu Trp Met Val Leu Asp Ala Ile Pro Ile Ile 220 225 230			723
cca cct gaa ctc cgc cca atg gta caa cta gaa ggt ggc cgg ttt gca Pro Pro Glu Leu Arg Pro Met Val Gln Leu Glu Gly Gly Arg Phe Ala 235 240 245 250			771
acc agc gac ttg aac gac ttg tac cgc cgg gtg att aac cgg aac aac Thr Ser Asp Leu Asn Asp Leu Tyr Arg Arg Val Ile Asn Arg Asn Asn 255 260 265			819
cgg ttg aaa cgc ttg ctt gac ttg aat gcc ccc cac att atc gtc caa Arg Leu Lys Arg Leu Leu Asp Leu Asn Ala Pro His Ile Ile Val Gln 270 275 280			867

aat gaa aaa cgg atg ctg caa gaa gct gtt gac gcc ttg att gac aat Asn Glu Lys Arg Met Leu Gln Glu Ala Val Asp Ala Leu Ile Asp Asn 285 290 295	915
ggc cgt cgc ggt cgg gca gtc aac ggt cct ggt aac cgt ccg ctt aaa Gly Arg Arg Gly Arg Ala Val Asn Gly Pro Gly Asn Arg Pro Leu Lys 300 305 310	963
tct ctt tct cac atg ttg aaa ggg aaa caa ggg cgc ttc cgt cag aac Ser Leu Ser His Met Leu Lys Gly Lys Gln Gly Arg Phe Arg Gln Asn 315 320 325 330	1011
cta cta ggg aaa cgg gtt gac tac tct ggc cgg tct gtc att gtt gtt Leu Leu Gly Lys Arg Val Asp Tyr Ser Gly Arg Ser Val Ile Val Val 335 340 345	1059
ggg cca acc ctt aaa atg tac caa tgt ggt cta ccg aaa gaa atg gcc Gly Pro Thr Leu Lys Met Tyr Gln Cys Gly Leu Pro Lys Glu Met Ala 350 355 360	1107
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ctc tta aac cgg gcc cct acc ctt cac cgg cta ggg atc caa gcc ttt Leu Leu Asn Arg Ala Pro Thr Leu His Arg Leu Gly Ile Gln Ala Phe 415 420 425	1299
gaa cct gtc ctt gtc aat ggg aag gct att cgc tta cac cca ctc gct Glu Pro Val Leu Val Asn Gly Lys Ala Ile Arg Leu His Pro Leu Ala 430 435 440	1347
tgt gaa gcc tac aat gct gac ttt gac gga gac caa atg gct gtc cac Cys Glu Ala Tyr Asn Ala Asp Phe Asp Gly Asp Gln Met Ala Val His 445 450 455	1395
gta ccc ctc agt gat gaa gcc cag gca gaa gcc cgc atc tta atg ctg Val Pro Leu Ser Asp Glu Ala Gln Ala Glu Ala Arg Ile Leu Met Leu 460 465 470	1443
ggc gcc caa aat atc tta aac cct aaa gat ggt caa cca gtc gtt acc Gly Ala Gln Asn Ile Leu Asn Pro Lys Asp Gly Gln Pro Val Val Thr 475 480 485 490	1491
cct tcc caa gac atg gtc cta ggg aac tac tac cta acc atg gaa gaa Pro Ser Gln Asp Met Val Leu Gly Asn Tyr Tyr Leu Thr Met Glu Glu 495 500 505	1539

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gac aag tac ttg att acc aca gtc ggt aag att atc ttt aat gaa att Asp Lys Tyr Leu Ile Thr Thr Val Gly Lys Ile Ile Phe Asn Glu Ile 555 560 565 570	1731
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gaa cag caa acc cca gac aag tac ttt gtc gac cgg ggc caa aac ttg Glu Gln Gln Thr Pro Asp Lys Tyr Phe Val Asp Arg Gly Gln Asn Leu 590 595 600	1827
aaa gac ctt att gcc gac cgt cct tta gtt cag cct ttc aaa aaa caa Lys Asp Leu Ile Ala Asp Arg Pro Leu Val Gln Pro Phe Lys Lys Gln 605 610 615	1875
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gaa acc tct aaa atg ttg gac cgc atg aag aac ttg ggc tac aag tac Glu Thr Ser Lys Met Leu Asp Arg Met Lys Asn Leu Gly Tyr Lys Tyr 635 640 645 650	1971
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gac aac gtt atc gat gtc tgg caa aag gct aag gat gaa att caa gat Asp Asn Val Ile Asp Val Trp Gln Lys Ala Lys Asp Glu Ile Gln Asp 700 705 710	2163
gcc ttg atg gat tcc ctt gac cca aga aat aac atc ttt atg atg tca Ala Leu Met Asp Ser Leu Asp Pro Arg Asn Asn Ile Phe Met Met Ser 715 720 725 730	2211
gac tct ggt gcc cgt ggg aat att tcc aac ttc acc caa cta gcc ggt	2259

Asp Ser Gly Ala Arg Gly Asn Ile Ser Asn Phe Thr Gln Leu Ala Gly	
735 740 745	
atg cgt ggt ttg atg gca gca cca agt ggt gag atc atg gaa ttg ccg	2307
Met Arg Gly Leu Met Ala Ala Pro Ser Gly Glu Ile Met Glu Leu Pro	
750 755 760	
atc acg tct aac ttc cgt gaa ggc ctg tct gtc tta gag atg ttt att	2355
Ile Thr Ser Asn Phe Arg Glu Gly Leu Ser Val Leu Glu Met Phe Ile	
765 770 775	
tcc acc cac ggt gcc cgt aaa ggc atg acc gat acc gcc ctt aaa act	2403
Ser Thr His Gly Ala Arg Lys Gly Met Thr Asp Thr Ala Leu Lys Thr	
780 785 790	
gcc gac tct ggt tac ttg acc aga cgt ttg gtt gat gtt gcc caa gac	2451
Ala Asp Ser Gly Tyr Leu Thr Arg Arg Leu Val Asp Val Ala Gln Asp	
795 800 805 810	
gtc atc atc cga gaa gaa gac tgt ggc act aaa cgt ggc ctt aaa gtt	2499
Val Ile Ile Arg Glu Glu Asp Cys Gly Thr Lys Arg Gly Leu Lys Val	
815 820 825	
tct gcc atc caa gta gga aat gaa cag att gaa agc ttg tct gac cgt	2547
Ser Ala Ile Gln Val Gly Asn Glu Gln Ile Glu Ser Leu Ser Asp Arg	
830 835 840	
atc ttg ggt cgt tat gcc caa gaa acc gtc acc cac ccc gaa act ggt	2595
Ile Leu Gly Arg Tyr Ala Gln Glu Thr Val Thr His Pro Glu Thr Gly	
845 850 855	
gaa gtc att gtt cac aag gat gaa ttg att gat gaa ggc aaa acc cga	2643
Glu Val Ile Val His Lys Asp Glu Leu Ile Asp Glu Gly Lys Thr Arg	
860 865 870	
aaa att gtc gat gcc ggt att gaa gaa gtt act atc cgg tct gcc ttc	2691
Lys Ile Val Asp Ala Gly Ile Glu Glu Val Thr Ile Arg Ser Ala Phe	
875 880 885 890	
tgc tgc aac acc aac cac ggt gtc tgc aag cac tgc tat ggc cgt aac	2739
Cys Cys Asn Thr Asn His Gly Val Cys Lys His Cys Tyr Gly Arg Asn	
895 900 905	
ttg gca act ggc cgg gaa gtt gaa gtt ggt gaa gca gtt gga act atc	2787
Leu Ala Thr Gly Arg Glu Val Glu Val Gly Glu Ala Val Gly Thr Ile	
910 915 920	
gct gcc caa tcc att ggg gaa ccc ggt acc caa ttg acc atg cgg acc	2835
Ala Ala Gln Ser Ile Gly Glu Pro Gly Thr Gln Leu Thr Met Arg Thr	
925 930 935	
ttc cac act ggt ggg gtc gct ggg gac gac atc acc caa ggt cta cca	2883
Phe His Thr Gly Gly Val Ala Gly Asp Asp Ile Thr Gln Gly Leu Pro	
940 945 950	
cgg gtt caa gaa atc ttt gaa gcc cgc cat ccg aaa ggg caa gcc acc	2931
Arg Val Gln Glu Ile Phe Glu Ala Arg His Pro Lys Gly Gln Ala Thr	

955	960	965	970	
att aca gaa gtg aat ggt caa atc caa gag atc gtt gaa gac cct gaa				2979
Ile Thr Glu Val Asn Gly Gln Ile Gln Glu Ile Val Glu Asp Pro Glu				
	975	980	985	
gaa cgc act aag acc gtc act gtt aag ggg aat gtt gac caa cgt gac				3027
Glu Arg Thr Lys Thr Val Thr Val Lys Gly Asn Val Asp Gln Arg Asp				
	990	995	1000	
tac tcc ttg cca atc aat gcc cgg atg aag gtt gaa gtt ggg gat tat				3075
Tyr Ser Leu Pro Ile Asn Ala Arg Met Lys Val Glu Val Gly Asp Tyr				
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gtt gaa cga ggc gat gct cta aac gag ggg tct att gat ccg aaa gag				3123
Val Glu Arg Gly Asp Ala Leu Asn Glu Gly Ser Ile Asp Pro Lys Glu				
	1020	1025	1030	
tta ctc gcg gtg agt gat atg atg aaa ttg cag aaa tac ctc ttg caa				3171
Leu Leu Ala Val Ser Asp Met Met Lys Leu Gln Lys Tyr Leu Leu Gln				
	1035	1040	1045	1050
gaa gtc caa tac gct tac cgg tct caa ggg gtc gaa att ggt gac aag				3219
Glu Val Gln Tyr Ala Tyr Arg Ser Gln Gly Val Glu Ile Gly Asp Lys				
	1055	1060	1065	
cac gtg gag gtt atg gtg cga caa atg ctc cgt aaa gtc cgt gtc ttg				3267
His Val Glu Val Met Val Arg Gln Met Leu Arg Lys Val Arg Val Leu				
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caa cca ggg gac act gat atc ctg cct ggt acc atg att gac ctc cac				3315
Gln Pro Gly Asp Thr Asp Ile Leu Pro Gly Thr Met Ile Asp Leu His				
	1085	1090	1095	
gac ttc aag gaa cgc aac caa gaa acc ttg atg tcc ggt ggc caa ccc				3363
Asp Phe Lys Glu Arg Asn Gln Glu Thr Leu Met Ser Gly Gly Gln Pro				
	1100	1105	1110	
gca act gct aga ctg gtc cta ctg ggt att acc aag gcc tcc ctt gaa				3411
Ala Thr Ala Arg Leu Val Leu Leu Gly Ile Thr Lys Ala Ser Leu Glu				
	1115	1120	1125	1130
acc aac tct ttc ttg tct gca gct tcc ttc caa gaa acc acc cgg gtc				3459
Thr Asn Ser Phe Leu Ser Ala Ala Ser Phe Gln Glu Thr Thr Arg Val				
	1135	1140	1145	
ctc acc gat gca gct att cgc ggt aaa gtt gat gac ctg gtt ggc ttg				3507
Leu Thr Asp Ala Ala Ile Arg Gly Lys Val Asp Asp Leu Val Gly Leu				
	1150	1155	1160	
aaa gaa aat gtt att atc ggt aaa tcc atc cca gct ggt act ggt atg				3555
Lys Glu Asn Val Ile Ile Gly Lys Ser Ile Pro Ala Gly Thr Gly Met				
	1165	1170	1175	
aga gcc tac agt aat att gaa cct aaa aaa gtt ggt gtc gtt agc gaa				3603
Arg Ala Tyr Ser Asn Ile Glu Pro Lys Lys Val Gly Val Val Ser Glu				
	1180	1185	1190	

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 Asn Val Tyr Ser Ile Asn Glu Glu Asp Gln Val Ser Gln Glu Glu Asn
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Glu Arg Ile Phe Gly Pro Thr Lys Asp Tyr Glu Cys Ala Cys Gly Lys
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Tyr Lys Arg Val His Tyr Lys Gly Ile Val Cys Asp Arg Cys Gly Val
 65 70 75 80

Glu Val Thr Lys Ser Ser Val Arg Arg Glu Arg Met Gly His Leu Glu
 85 90 95

Leu Ala Ala Pro Val Thr His Ile Trp Tyr Phe Lys Gly Ile Pro Ser
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Arg Met Gly Leu Ile Leu Asp Met Ser Pro Arg Ser Leu Glu Glu Ile
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Ile Tyr Phe Ala Ser Tyr Val Val Ile Asp Gly Gly Asp Thr Pro Leu
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Lys Tyr Gly Asn Glu Phe Gln Ala Glu Ile Gly Ala Glu Ala Val Arg

165

170

175

Thr Leu Leu Lys Asn Val Asp Leu Glu Gln Glu Val Ala Asp Leu Lys
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Glu Ile Leu Glu Thr Ala Thr Gly Gln Lys Arg Thr Arg Ala Ile Arg
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Arg Leu Asp Ile Ile Asp Ser Phe Lys Ser Ser Asn Asn Lys Pro Glu
210 215 220

Trp Met Val Leu Asp Ala Ile Pro Ile Ile Pro Pro Glu Leu Arg Pro
225 230 235 240

Met Val Gln Leu Glu Gly Gly Arg Phe Ala Thr Ser Asp Leu Asn Asp
245 250 255

Leu Tyr Arg Arg Val Ile Asn Arg Asn Asn Arg Leu Lys Arg Leu Leu
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Asp Leu Asn Ala Pro His Ile Ile Val Gln Asn Glu Lys Arg Met Leu
275 280 285

Gln Glu Ala Val Asp Ala Leu Ile Asp Asn Gly Arg Arg Gly Arg Ala
290 295 300

Val Asn Gly Pro Gly Asn Arg Pro Leu Lys Ser Leu Ser His Met Leu
305 310 315 320

Lys Gly Lys Gln Gly Arg Phe Arg Gln Asn Leu Leu Gly Lys Arg Val
325 330 335

Asp Tyr Ser Gly Arg Ser Val Ile Val Val Gly Pro Thr Leu Lys Met
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Tyr Gln Cys Gly Leu Pro Lys Glu Met Ala Ile Glu Leu Phe Lys Pro
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Phe Val Met Arg Glu Leu Val Glu Arg Asp Ile Ala Asn Asn Ile Lys
370 375 380

Asn Ala Lys Arg Lys Val Glu Arg Met Glu Asp Asp Val Trp Pro Val
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Leu Glu Asp Val Ile Lys Glu His Pro Val Leu Leu Asn Arg Ala Pro
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Thr Leu His Arg Leu Gly Ile Gln Ala Phe Glu Pro Val Leu Val Asn
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Gly Lys Ala Ile Arg Leu His Pro Leu Ala Cys Glu Ala Tyr Asn Ala
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Asp Phe Asp Gly Asp Gln Met Ala Val His Val Pro Leu Ser Asp Glu
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Asn Pro Lys Asp Gly Gln Pro Val Val Thr Pro Ser Gln Asp Met Val
485 490 495

Leu Gly Asn Tyr Tyr Leu Thr Met Glu Glu Glu Gly Lys Ile Gly Glu
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Gly Thr Val Phe Ser Ser Ala Ser Glu Ala Ile Gln Ala Tyr Gln Thr
515 520 525

Gly Tyr Val His Leu His Thr Arg Val Ala Ile Arg Ala Val Asp Leu
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Pro Asp Lys Pro Phe Thr Asp Trp Gln Lys Asp Lys Tyr Leu Ile Thr
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580 585 590

Lys Tyr Phe Val Asp Arg Gly Gln Asn Leu Lys Asp Leu Ile Ala Asp
595 600 605

Arg Pro Leu Val Gln Pro Phe Lys Lys Gln Asp Leu Ser Asn Ile Ile
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Ala Glu Val Phe Asn Asn Phe Gln Val Thr Glu Thr Ser Lys Met Leu
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Asp Arg Met Lys Asn Leu Gly Tyr Lys Tyr Ser Thr Arg Ser Gly Ile
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Thr Val Gly Ile Ala Asp Val Ser Val Leu Glu Ala Lys Pro Glu Ile
660 665 670

Leu Lys Glu Ala His Ala Lys Val Asp Lys Ile Asn Ala Thr His Arg
675 680 685

Arg Gly Leu Ile Thr Glu Glu Glu Arg Tyr Asp Asn Val Ile Asp Val
690 695 700

Trp Gln Lys Ala Lys Asp Glu Ile Gln Asp Ala Leu Met Asp Ser Leu
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Asp Pro Arg Asn Asn Ile Phe Met Met Ser Asp Ser Gly Ala Arg Gly
725 730 735

Asn Ile Ser Asn Phe Thr Gln Leu Ala Gly Met Arg Gly Leu Met Ala
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Ala Pro Ser Gly Glu Ile Met Glu Leu Pro Ile Thr Ser Asn Phe Arg
755 760 765

Glu Gly Leu Ser Val Leu Glu Met Phe Ile Ser Thr His Gly Ala Arg
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Lys Gly Met Thr Asp Thr Ala Leu Lys Thr Ala Asp Ser Gly Tyr Leu
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805 810 815

Asp Cys Gly Thr Lys Arg Gly Leu Lys Val Ser Ala Ile Gln Val Gly
820 825 830

Asn Glu Gln Ile Glu Ser Leu Ser Asp Arg Ile Leu Gly Arg Tyr Ala
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Gln Glu Thr Val Thr His Pro Glu Thr Gly Glu Val Ile Val His Lys
850 855 860

Asp Glu Leu Ile Asp Glu Gly Lys Thr Arg Lys Ile Val Asp Ala Gly
865 870 875 880

Ile Glu Glu Val Thr Ile Arg Ser Ala Phe Cys Cys Asn Thr Asn His
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Gly Val Cys Lys His Cys Tyr Gly Arg Asn Leu Ala Thr Gly Arg Glu
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Glu Pro Gly Thr Gln Leu Thr Met Arg Thr Phe His Thr Gly Gly Val
930 935 940

Ala Gly Asp Asp Ile Thr Gln Gly Leu Pro Arg Val Gln Glu Ile Phe
945 950 955 960

Glu Ala Arg His Pro Lys Gly Gln Ala Thr Ile Thr Glu Val Asn Gly
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Gln Ile Gln Glu Ile Val Glu Asp Pro Glu Glu Arg Thr Lys Thr Val
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Thr Val Lys Gly Asn Val Asp Gln Arg Asp Tyr Ser Leu Pro Ile Asn
995 1000 1005

Ala Arg Met Lys Val Glu Val Gly Asp Tyr Val Glu Arg Gly Asp Ala
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Leu Asn Glu Gly Ser Ile Asp Pro Lys Glu Leu Leu Ala Val Ser Asp
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Met Met Lys Leu Gln Lys Tyr Leu Leu Gln Glu Val Gln Tyr Ala Tyr
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Arg Gln Met Leu Arg Lys Val Arg Val Leu Gln Pro Gly Asp Thr Asp

1075

1080

1085

Ile Leu Pro Gly Thr Met Ile Asp Leu His Asp Phe Lys Glu Arg Asn
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Asp	Pro	Glu	Ala	Gly	Glu	Val	Leu	Ala	Glu	Glu	Gly	Ser	Glu	Val	Thr	
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cgg	tct	gtg	atg	gac	aag	ctt	ggc	cct	tac	ctt	gac	ggg	gac	atg	aac	1008
Arg	Ser	Val	Met	Asp	Lys	Leu	Gly	Pro	Tyr	Leu	Asp	Gly	Asp	Met	Asn	
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Gln	Val	Thr	Ile	Asn	Pro	Ser	Glu	Glu	Ala	Val	Ile	Pro	Glu	Pro	Ile	
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Val	Asn	Met	Ile	Gly	Asn	Gly	His	Pro	Asp	Lys	Lys	Ala	Lys	Trp	Ile	
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Thr	Pro	Ala	Asp	Met	Ile	Ala	Ala	Met	Ser	Tyr	Phe	Phe	Asn	Leu	Gln	
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gaa	ggc	att	ggc	gat	gtt	gac	gat	atc	gac	cac	ttg	ggg	aac	cgt	cgg	1248
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atc	cgg	tca	gtc	gga	gag	ctt	ttg	caa	aac	caa	ttc	cga	att	ggg	ctc	1296
Ile	Arg	Ser	Val	Gly	Glu	Leu	Leu	Gln	Asn	Gln	Phe	Arg	Ile	Gly	Leu	
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Ser	Arg	Met	Glu	Arg	Val	Val	Arg	Glu	Arg	Met	Ser	Ile	Gln	Asp	Ile	
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Ser	Ser	Thr	Thr	Pro	Gln	Gln	Leu	Ile	Asn	Ile	Arg	Pro	Val	Val	Ala	
		450					455					460				
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Ser	Leu	Lys	Glu	Phe	Phe	Gly	Ser	Ser	Gln	Leu	Ser	Gln	Phe	Met	Asp	
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Asp Val His Tyr Ser His Tyr Gly Arg Met Cys Pro Ile Glu Thr Pro				
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Ile Asn Lys Phe Gly Phe Ile Glu Thr Pro Tyr Arg Arg Val Asp Arg				
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Glu Asp Leu Tyr Val Val Ala Gln Ala Asn Ala Glu Leu Asp Glu Asp				
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Gly His Phe Ala Asn Asp Val Val Leu Ala Arg Arg Arg Asp Val Asn				
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Glu Glu Val Asp Ala Ser Glu Val Asp Tyr Met Asp Val Ser Pro Lys				
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Gln Val Val Ser Val Ala Thr Ala Ser Ile Pro Phe Leu Glu Asn Asp				
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Asp Ser Asn Arg Ala Leu Met Gly Ala Asn Met Gln Arg Gln Ala Val				
640		645	650	655
cct ctt atg caa cca gag tcc cca cta gta gga act gga atc gaa cac				2016
Pro Leu Met Gln Pro Glu Ser Pro Leu Val Gly Thr Gly Ile Glu His				
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att gca gcc cgt gac tct gga gct gcc gtt att gcc aag gct gac ggg				2064
Ile Ala Ala Arg Asp Ser Gly Ala Ala Val Ile Ala Lys Ala Asp Gly				
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gcc ggc gac gaa ctg tca cca ggc gtt aac tac ctt gtc cga gtt ttc Ala Gly Asp Glu Leu Ser Pro Gly Val Asn Tyr Leu Val Arg Val Phe 900 905 910	2736
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gga agg gta gac acc tat gaa gcc att gtc aag ggc caa cgc att cca Gly Arg Val Asp Thr Tyr Glu Ala Ile Val Lys Gly Gln Arg Ile Pro 1105 1110 1115	3360
aaa cct ggt gta cct gaa tcc ttc cgt gtc ctc gtg aaa gaa ctc cag Lys Pro Gly Val Pro Glu Ser Phe Arg Val Leu Val Lys Glu Leu Gln 1120 1125 1130 1135	3408
tct ctg ggg ttg gac ctg aaa gtc ctc gac aag gaa caa aac gaa atc Ser Leu Gly Leu Asp Leu Lys Val Leu Asp Lys Glu Gln Asn Glu Ile 1140 1145 1150	3456
aat ctc aag gct gaa gat gac gag tcg gaa gac caa gtc gtt gat tcc Asn Leu Lys Ala Glu Asp Asp Glu Ser Glu Asp Gln Val Val Asp Ser 1155 1160 1165	3504
cta gaa gaa atg cgt aaa gag cag gaa gaa gaa cgc cgt aag gaa aaa	3552

Leu Glu Glu Met Arg Lys Glu Gln Glu Glu Glu Arg Arg Lys Glu Lys
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 Glu Lys Glu Glu Pro Ser Thr Glu Ser
 1185 1190

3582

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<211> 1192

<212> PRT

<213> Alloiococcus otitidis

<400> 14

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 20 25 30

Ile Glu Ile Gln Thr Asp Ser Tyr Asp Trp Phe Leu Asp Glu Gly Leu
 35 40 45

Lys Glu Met Phe Ser Asp Ile Ser Pro Ile Asp Asp Phe Ser Gly Asn
 50 55 60

Leu Ser Leu Glu Phe Val Asp Tyr Lys Phe Tyr Glu Ser Lys Tyr Thr
 65 70 75 80

Val Glu Glu Ala Arg Glu His Asp Ala Asn Tyr Ser Ala Pro Leu Tyr
 85 90 95

Val Lys Leu Arg Leu Ile Asn Lys Glu Thr Gly Glu Val Lys Glu Gln
 100 105 110

Glu Val Phe Phe Gly Asp Phe Pro Leu Met Thr Glu Gln Gly Thr Phe
 115 120 125

Ile Ile Asn Gly Ala Glu Arg Val Ile Val Ser Gln Leu Val Arg Ser
 130 135 140

Pro Gly Val Tyr Tyr Ser Pro Lys Val Glu Lys Asn Gly Arg Glu Gly
 145 150 155 160

Phe Ser Thr Val Leu Ile Pro Asn Arg Gly Ala Trp Leu Glu Tyr Glu
 165 170 175

Thr Asp Thr Lys Gly Ile Ser Asn Val Arg Ile Asp Arg Thr Arg Lys
180 185 190

Ile Pro Ile Thr Val Leu Leu Arg Ala Leu Gly Ile Gly Ser Asp Asp
195 200 205

Glu Ile Ile Asp Leu Ile Gly Leu Asn Asp Ser Leu Glu Ala Thr Leu
210 215 220

Glu Lys Asp Val His Lys Ser Thr Ser Asp Ser Arg Val Glu Glu Ala
225 230 235 240

Leu Lys Asp Leu Tyr Glu Arg Leu Arg Pro Gly Glu Pro Lys Thr Ala
245 250 255

Glu Ser Ser Arg Asn Leu Ile Asn Thr Arg Phe Phe Asp His Lys Arg
260 265 270

Tyr Asp Leu Ala Tyr Val Gly Arg Tyr Lys Met Asn Lys Lys Leu Asp
275 280 285

Leu Lys Thr Arg Leu Met Gly Thr Val Leu Ala Glu Asn Leu Val Asp
290 295 300

Pro Glu Ala Gly Glu Val Leu Ala Glu Glu Gly Ser Glu Val Thr Arg
305 310 315 320

Ser Val Met Asp Lys Leu Gly Pro Tyr Leu Asp Gly Asp Met Asn Gln
325 330 335

Val Thr Ile Asn Pro Ser Glu Glu Ala Val Ile Pro Glu Pro Ile Asp
340 345 350

Leu Gln Ile Val Lys Val Tyr Ser Lys Glu Asp Pro Asp Arg Ile Val
355 360 365

Asn Met Ile Gly Asn Gly His Pro Asp Lys Lys Ala Lys Trp Ile Thr
370 375 380

Pro Ala Asp Met Ile Ala Ala Met Ser Tyr Phe Phe Asn Leu Gln Glu
385 390 395 400

Gly Ile Gly Asp Val Asp Asp Ile Asp His Leu Gly Asn Arg Arg Ile
405 410 415

Arg Ser Val Gly Glu Leu Leu Gln Asn Gln Phe Arg Ile Gly Leu Ser
420 425 430

Arg Met Glu Arg Val Val Arg Glu Arg Met Ser Ile Gln Asp Ile Ser
435 440 445

Ser Thr Thr Pro Gln Gln Leu Ile Asn Ile Arg Pro Val Val Ala Ser
450 455 460

Leu Lys Glu Phe Phe Gly Ser Ser Gln Leu Ser Gln Phe Met Asp Gln
465 470 475 480

Thr Asn Pro Leu Gly Glu Leu Thr His Lys Arg Arg Leu Ser Ala Leu
485 490 495

Gly Pro Gly Gly Leu Thr Arg Asp Arg Ala Gly Tyr Glu Val Arg Asp
500 505 510

Val His Tyr Ser His Tyr Gly Arg Met Cys Pro Ile Glu Thr Pro Glu
515 520 525

Gly Pro Asn Ile Gly Leu Ile Asn Ser Leu Ser Thr Tyr Ala Lys Ile
530 535 540

Asn Lys Phe Gly Phe Ile Glu Thr Pro Tyr Arg Arg Val Asp Arg Glu
545 550 555 560

Thr Gly Gln Val Thr Asp Lys Ile Asp Tyr Leu Thr Ala Asp Glu Glu
565 570 575

Asp Leu Tyr Val Val Ala Gln Ala Asn Ala Glu Leu Asp Glu Asp Gly
580 585 590

His Phe Ala Asn Asp Val Val Leu Ala Arg Arg Arg Asp Val Asn Glu
595 600 605

Glu Val Asp Ala Ser Glu Val Asp Tyr Met Asp Val Ser Pro Lys Gln
610 615 620

Val Val Ser Val Ala Thr Ala Ser Ile Pro Phe Leu Glu Asn Asp Asp

625 630 635 640

Ser Asn Arg Ala Leu Met Gly Ala Asn Met Gln Arg Gln Ala Val Pro
645 650 655

Leu Met Gln Pro Glu Ser Pro Leu Val Gly Thr Gly Ile Glu His Ile
660 665 670

Ala Ala Arg Asp Ser Gly Ala Ala Val Ile Ala Lys Ala Asp Gly Val
675 680 685

Val Glu Tyr Val Asp Ala Lys Thr Val Lys Val Arg Gln Ala Asp Gly
690 695 700

Thr Leu Asn Asn Tyr Lys Leu Ala Lys Tyr Lys Arg Ser Asn Ser Gly
705 710 715 720

Thr Ser Tyr Asn Gln Arg Pro Ile Val Lys Thr Gly Glu Glu Val Asp
725 730 735

Lys Gly Asp Ile Leu Ala Asp Gly Pro Ser Met Glu Asn Gly Glu Met
740 745 750

Ala Leu Gly Lys Asn Pro Leu Ile Ala Phe Thr Thr Phe Asp Gly Tyr
755 760 765

Asn Phe Glu Asp Ala Val Ile Met Ser Glu Arg Leu Val Lys Asp Asp
770 775 780

Val Tyr Thr Ser Ile His Ile Glu Glu Tyr Glu Ser Glu Ala Arg Asp
785 790 795 800

Thr Lys Leu Gly Pro Glu Glu Ile Thr Arg Glu Ile Pro Asn Val Gly
805 810 815

Glu Ser Ala Leu Lys Asn Leu Asp Glu Arg Gly Ile Ile Arg Ile Gly
820 825 830

Ala Glu Val Arg Asp Gly Asp Ile Leu Val Gly Lys Val Thr Pro Lys
835 840 845

Gly Val Ser Glu Leu Ser Ala Glu Glu Lys Leu Leu His Ala Ile Phe
850 855 860

Gly Glu Lys Ala Arg Glu Val Arg Asp Thr Ser Leu Arg Val Pro His
865 870 875 880

Gly Ser Gly Gly Ile Val His Asp Val Gln Ile Phe Thr Arg Glu Ala
885 890 895

Gly Asp Glu Leu Ser Pro Gly Val Asn Tyr Leu Val Arg Val Phe Ile
900 905 910

Ala Gln Lys Arg Lys Ile Asp Val Gly Asp Lys Met Ala Gly Arg His
915 920 925

Gly Asn Lys Gly Val Val Ser Leu Ile Leu Pro Glu Glu Asp Met Pro
930 935 940

Phe Met Pro Asp Gly Thr Pro Ile Asp Ile Met Leu Asn Pro Leu Gly
945 950 955 960

Val Pro Ser Arg Met Asn Val Gly Gln Val Ile Glu Leu His Met Gly
965 970 975

Met Ala Ala Arg Gln Leu Gly Glu His Ile Ala Thr Pro Val Phe Asp
980 985 990

Gly Ala Asn Glu Glu Asp Val Trp Glu Thr Ile Lys Glu Ala Gly Met
995 1000 1005

Asp Ala Asp Ala Lys Thr Val Leu Tyr Asp Gly Arg Thr Gly Glu Pro
1010 1015 1020

Phe Asp Asn Lys Val Ser Val Gly Val Met Tyr Phe Ile Lys Leu Val
1025 1030 1035 1040

His Met Val Asp Asp Lys Leu His Ala Arg Ser Thr Gly Pro Tyr Ser
1045 1050 1055

Leu Val Thr Gln Gln Pro Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln
1060 1065 1070

Arg Phe Gly Glu Met Glu Val Trp Ala Leu Glu Ala Tyr Gly Ala Ser
1075 1080 1085

Arg Thr Leu Gln Glu Ile Leu Thr Tyr Lys Ser Asp Asp Val Ile Gly
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Arg Val Asp Thr Tyr Glu Ala Ile Val Lys Gly Gln Arg Ile Pro Lys
 1105 1110 1115 1120

Pro Gly Val Pro Glu Ser Phe Arg Val Leu Val Lys Glu Leu Gln Ser
 1125 1130 1135

Leu Gly Leu Asp Leu Lys Val Leu Asp Lys Glu Gln Asn Glu Ile Asn
 1140 1145 1150

Leu Lys Ala Glu Asp Asp Glu Ser Glu Asp Gln Val Val Asp Ser Leu
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Glu Glu Met Arg Lys Glu Gln Glu Glu Glu Arg Arg Lys Glu Lys Glu
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Lys Glu Glu Pro Ser Thr Glu Ser
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<210> 15
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 <212> DNA
 <213> *Alloioicoccus otitidis*

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ctg cca cca caa aat att gaa gcg gag caa tcc gtc tta ggg tcc gtc 99
 Leu Pro Pro Gln Asn Ile Glu Ala Glu Gln Ser Val Leu Gly Ser Val
 10 15 20 25

ctc tta aat gca gaa gcc ttg gtg gcg gcc atg gaa tat gtg gat gaa 147
 Leu Leu Asn Ala Glu Ala Leu Val Ala Ala Met Glu Tyr Val Asp Glu
 30 35 40

gat gac ttt tac cgg cgg gcc cac cag ttg atc ttt aag gcc atg ata 195
 Asp Asp Phe Tyr Arg Arg Ala His Gln Leu Ile Phe Lys Ala Met Ile
 45 50 55

gac ctc tat gaa gac aac cag gcc att gat gtc att acc att aaa gac 243

Asp	Leu	Tyr	Glu	Asp	Asn	Gln	Ala	Ile	Asp	Val	Ile	Thr	Ile	Lys	Asp		
	60						65					70					
aag	ctg	gaa	gcc	aat	gac	cag	ttg	gag	gat	atc	ggg	ggt	gcc	tct	tac		291
Lys	Leu	Glu	Ala	Asn	Asp	Gln	Leu	Glu	Asp	Ile	Gly	Gly	Ala	Ser	Tyr		
	75					80					85						
cta	gct	gag	att	gct	ggg	gtc	acc	cca	acc	gca	gct	aac	gtg	tcc	tat		339
Leu	Ala	Glu	Ile	Ala	Gly	Val	Thr	Pro	Thr	Ala	Ala	Asn	Val	Ser	Tyr		
	90				95					100					105		
tac	gct	aag	att	gtg	gaa	gat	cgg	tct	ctt	ttg	cgc	aac	ttg	att	gcg		387
Tyr	Ala	Lys	Ile	Val	Glu	Asp	Arg	Ser	Leu	Leu	Arg	Asn	Leu	Ile	Ala		
				110					115					120			
aca	gct	aat	gag	att	gcc	cag	tct	ggc	tac	gaa	gac	cat	gac	gat	gtg		435
Thr	Ala	Asn	Glu	Ile	Ala	Gln	Ser	Gly	Tyr	Glu	Asp	His	Asp	Asp	Val		
				125				130					135				
cca	gaa	gtt	tta	aac	aat	gct	gag	cag	aag	atc	ttg	cag	gtt	tct	gaa		483
Pro	Glu	Val	Leu	Asn	Asn	Ala	Glu	Gln	Lys	Ile	Leu	Gln	Val	Ser	Glu		
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aaa	cga	aac	cgg	acc	ggc	ttt	gct	agt	att	tca	gaa	atc	ctc	cac	caa		531
Lys	Arg	Asn	Arg	Thr	Gly	Phe	Ala	Ser	Ile	Ser	Glu	Ile	Leu	His	Gln		
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acc	atc	gag	cat	att	gat	gaa	ctc	cac	caa	agg	gat	gaa	gag	atc	acc		579
Thr	Ile	Glu	His	Ile	Asp	Glu	Leu	His	Gln	Arg	Asp	Glu	Glu	Ile	Thr		
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Gly	Ile	Ser	Thr	Gly	Tyr	Pro	Tyr	Leu	Asp	Arg	Met	Thr	Ser	Gly	Leu		
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His	Glu	Asp	Glu	Leu	Ile	Ile	Val	Ala	Ala	Arg	Pro	Gly	Val	Gly	Lys		
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acg	gct	ttt	gcc	ttg	aat	gtc	gcc	caa	aat	atc	ggg	aca	gcc	aca	gat		723
Thr	Ala	Phe	Ala	Leu	Asn	Val	Ala	Gln	Asn	Ile	Gly	Thr	Ala	Thr	Asp		
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gaa	act	att	gcg	att	ttt	tcc	ctt	gag	atg	ggg	gct	gaa	cag	ctg	gtc		771
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aac	cgg	atg	tta	tgt	tca	gaa	ggc	agt	att	gat	gcc	act	aac	ctc	cga		819
Asn	Arg	Met	Leu	Cys	Ser	Glu	Gly	Ser	Ile	Asp	Ala	Thr	Asn	Leu	Arg		
	250				255					260					265		
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Asn	Gly	Lys	Leu	Thr	Pro	Glu	Glu	Tyr	Asp	Arg	Leu	Phe	Val	Ala	Met		
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ggg	agc	ttg	tct	gaa	gct	gat	att	tat	att	gat	gac	act	ccc	ggc	atc		915
Gly	Ser	Leu	Ser	Glu	Ala	Asp	Ile	Tyr	Ile	Asp	Asp	Thr	Pro	Gly	Ile		

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gga agt ctg ggc ttg att gtc att gac tac ctg caa ttg atc gaa gga Gly Ser Leu Gly Leu Ile Val Ile Asp Tyr Leu Gln Leu Ile Glu Gly 315 320 325			1011
gct tca aac tat gaa tcc aga cag cag cag gtg tct gat ata tct cgg Ala Ser Asn Tyr Glu Ser Arg Gln Gln Gln Val Ser Asp Ile Ser Arg 330 335 340 345			1059
cag ctg aag aag ctt tct aag gaa ctt tct gtc cca gtt att gcc ctg Gln Leu Lys Lys Leu Ser Lys Glu Leu Ser Val Pro Val Ile Ala Leu 350 355 360			1107
tca caa ctg tcc cgg agt gtg gaa cag aga caa gac aag cgg ccc atc Ser Gln Leu Ser Arg Ser Val Glu Gln Arg Gln Asp Lys Arg Pro Ile 365 370 375			1155
ctc agt gac ttg cgg gaa tca ggg tcg att gaa cag gat gcc gat att Leu Ser Asp Leu Arg Glu Ser Gly Ser Ile Glu Gln Asp Ala Asp Ile 380 385 390			1203
gtg gcc ttc ctt tac cgg gag gac tac tac caa aat gaa gaa gat atc Val Ala Phe Leu Tyr Arg Glu Asp Tyr Tyr Gln Asn Glu Glu Asp Ile 395 400 405			1251
gat gag gac ttt gtc gat aat agc gtg gaa gtc att atc gaa aaa aac Asp Glu Asp Phe Val Asp Asn Ser Val Glu Val Ile Ile Glu Lys Asn 410 415 420 425			1299
cgg tca gga gct cga gga aca gtc aag ttg aac ttt aag aaa gag ttc Arg Ser Gly Ala Arg Gly Thr Val Lys Leu Asn Phe Lys Lys Glu Phe 430 435 440			1347
aac aaa ttt acc tcg att tct tac cgg tct gaa gat gaa gtc cca gcc Asn Lys Phe Thr Ser Ile Ser Tyr Arg Ser Glu Asp Glu Val Pro Ala 445 450 455			1395
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<211> 460

<212> PRT

<213> Alloiococcus otitidis

<400> 16

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Val Ala Ala Met Glu Tyr Val Asp Glu Asp Asp Phe Tyr Arg Arg Ala
35 40 45

His Gln Leu Ile Phe Lys Ala Met Ile Asp Leu Tyr Glu Asp Asn Gln
50 55 60

Ala Ile Asp Val Ile Thr Ile Lys Asp Lys Leu Glu Ala Asn Asp Gln
65 70 75 80

Leu Glu Asp Ile Gly Gly Ala Ser Tyr Leu Ala Glu Ile Ala Gly Val
85 90 95

Thr Pro Thr Ala Ala Asn Val Ser Tyr Tyr Ala Lys Ile Val Glu Asp
100 105 110

Arg Ser Leu Leu Arg Asn Leu Ile Ala Thr Ala Asn Glu Ile Ala Gln
115 120 125

Ser Gly Tyr Glu Asp His Asp Asp Val Pro Glu Val Leu Asn Asn Ala
130 135 140

Glu Gln Lys Ile Leu Gln Val Ser Glu Lys Arg Asn Arg Thr Gly Phe
145 150 155 160

Ala Ser Ile Ser Glu Ile Leu His Gln Thr Ile Glu His Ile Asp Glu
165 170 175

Leu His Gln Arg Asp Glu Glu Ile Thr Gly Ile Ser Thr Gly Tyr Pro
180 185 190

Tyr Leu Asp Arg Met Thr Ser Gly Leu His Glu Asp Glu Leu Ile Ile
195 200 205

Val Ala Ala Arg Pro Gly Val Gly Lys Thr Ala Phe Ala Leu Asn Val
210 215 220

Ala Gln Asn Ile Gly Thr Ala Thr Asp Glu Thr Ile Ala Ile Phe Ser
225 230 235 240

Leu Glu Met Gly Ala Glu Gln Leu Val Asn Arg Met Leu Cys Ser Glu

245

250

255

Gly Ser Ile Asp Ala Thr Asn Leu Arg Asn Gly Lys Leu Thr Pro Glu
260 265 270

Glu Tyr Asp Arg Leu Phe Val Ala Met Gly Ser Leu Ser Glu Ala Asp
275 280 285

Ile Tyr Ile Asp Asp Thr Pro Gly Ile Arg Thr Ala Glu Ile Arg Ala
290 295 300

Lys Cys Arg Arg Leu Val Gln Glu Lys Gly Ser Leu Gly Leu Ile Val
305 310 315 320

Ile Asp Tyr Leu Gln Leu Ile Glu Gly Ala Ser Asn Tyr Glu Ser Arg
325 330 335

Gln Gln Gln Val Ser Asp Ile Ser Arg Gln Leu Lys Lys Leu Ser Lys
340 345 350

Glu Leu Ser Val Pro Val Ile Ala Leu Ser Gln Leu Ser Arg Ser Val
355 360 365

Glu Gln Arg Gln Asp Lys Arg Pro Ile Leu Ser Asp Leu Arg Glu Ser
370 375 380

Gly Ser Ile Glu Gln Asp Ala Asp Ile Val Ala Phe Leu Tyr Arg Glu
385 390 395 400

Asp Tyr Tyr Gln Asn Glu Glu Asp Ile Asp Glu Asp Phe Val Asp Asn
405 410 415

Ser Val Glu Val Ile Ile Glu Lys Asn Arg Ser Gly Ala Arg Gly Thr
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Val Lys Leu Asn Phe Lys Lys Glu Phe Asn Lys Phe Thr Ser Ile Ser
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Tyr Arg Ser Glu Asp Glu Val Pro Ala Asn Phe Gly
450 455 460

<210> 17

<211> 2484

<212> DNA

<213> *Alloioioccus otitidis*

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<221> CDS

<222> (10)..(2484)

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Leu	Ser	Lys	Glu	Met	Lys	Asn	Ser	Phe	Leu	Asp	Tyr	Ala	Met	Ser	Val	
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cac	cga	aga	atc	ctg	tac	gga	atg	aat	gaa	ctg	ggc	tta	acc	ccg	gac	195
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Lys	Ser	Tyr	Lys	Lys	Ser	Ala	Arg	Ile	Val	Gly	Asp	Val	Met	Gly	Lys	
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Tyr	His	Pro	His	Gly	Asp	Thr	Ala	Ile	Tyr	Asp	Ser	Met	Val	Arg	Met	
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Ala	Gln	Asp	Phe	Ser	Tyr	Arg	Val	Pro	Leu	Val	Asp	Gly	His	Gly	Asn	
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Phe	Gly	Ser	Val	Asp	Gly	Asp	Gly	Ala	Ala	Ala	Met	Arg	Tyr	Thr	Glu	
				115				120							125	
gcc	cgg	atg	tcc	aag	atg	gcc	ttg	gaa	ctc	ctg	cga	gac	atc	aac	aag	435
Ala	Arg	Met	Ser	Lys	Met	Ala	Leu	Glu	Leu	Leu	Arg	Asp	Ile	Asn	Lys	
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gat	acc	att	gac	tac	cac	gat	aac	tat	gat	ggg	act	gag	tcg	gaa	ccc	483
Asp	Thr	Ile	Asp	Tyr	His	Asp	Asn	Tyr	Asp	Gly	Thr	Glu	Ser	Glu	Pro	
		145					150					155				
gat	atc	ctt	cct	gcc	cgc	ttc	ccc	aac	ctc	tta	gtc	aac	ggg	gct	tcg	531
Asp	Ile	Leu	Pro	Ala	Arg	Phe	Pro	Asn	Leu	Leu	Val	Asn	Gly	Ala	Ser	
	160					165					170					
ggg	att	gct	gtt	ggg	atg	gca	acc	aac	atc	cca	ccc	cac	aac	ctt	aag	579
Gly	Ile	Ala	Val	Gly	Met	Ala	Thr	Asn	Ile	Pro	Pro	His	Asn	Leu	Lys	
175					180					185					190	

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gtg gct gac ctt atg gaa gtc tta cca gga cct gac ttt ccg act ggg Val Ala Asp Leu Met Glu Val Leu Pro Gly Pro Asp Phe Pro Thr Gly 210 215 220	675
gct tcc ctt att ggt gtt tct ggc gtc cgc aag gct tat gag acc ggt Ala Ser Leu Ile Gly Val Ser Gly Val Arg Lys Ala Tyr Glu Thr Gly 225 230 235	723
cgt ggg tcc att aaa tta cgg gcc aag tcc cgg atc gat gtc gac caa Arg Gly Ser Ile Lys Leu Arg Ala Lys Ser Arg Ile Asp Val Asp Gln 240 245 250	771
aaa ggt aag gaa aga att att atc gac gaa att cct tac atg gtc aac Lys Gly Lys Glu Arg Ile Ile Ile Asp Glu Ile Pro Tyr Met Val Asn 255 260 265 270	819
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cgg att gtg atc gat gta cgc cgg gat act tct gct ggt ata tta ctt Arg Ile Val Ile Asp Val Arg Arg Asp Thr Ser Ala Gly Ile Leu Leu 305 310 315	963
aac aag ctt tac aaa atg acc caa ttg cag gtt tct ttt ggc ttt aac Asn Lys Leu Tyr Lys Met Thr Gln Leu Gln Val Ser Phe Gly Phe Asn 320 325 330	1011
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ggg ctt cgg act gcc tta gac cat atc gat gcc att att acc att atc Gly Leu Arg Thr Ala Leu Asp His Ile Asp Ala Ile Ile Thr Ile Ile 385 390 395	1203
cgt cag tcc cag caa gct gaa gaa gcc aaa agt caa ttg atg gct tct Arg Gln Ser Gln Gln Ala Glu Glu Ala Lys Ser Gln Leu Met Ala Ser 400 405 410	1251
tat gac ctc tct gac cgt caa gcc cag gcg att tta gac atg cgg atg	1299

ggg ttg aac gcg atc aat cta aat gaa ggc gat gaa ttg gtt aac gtg 1971
Gly Leu Asn Ala Ile Asn Leu Asn Glu Gly Asp Glu Leu Val Asn Val

640	645	650	
gtc cct acc cac aat gac cag gcc att atc ctg gcc agc cag caa ggc Val Pro Thr His Asn Asp Gln Ala Ile Ile Leu Ala Ser Gln Gln Gly 655 660 665 670			2019
tat gcg gtc tac ttt gat gaa aaa gat atc cgt agc atg ggt cga ggg Tyr Ala Val Tyr Phe Asp Glu Lys Asp Ile Arg Ser Met Gly Arg Gly 675 680 685			2067
gct gca ggt gtc cgt gga att cgc tta ggt gat gcc gac aca gtg gtt Ala Ala Gly Val Arg Gly Ile Arg Leu Gly Asp Gly Asp Thr Val Val 690 695 700			2115
gcc atg gaa gtc tta gag ccg gcc caa gac gta tta gtc att act gaa Ala Met Glu Val Leu Glu Pro Gly Gln Asp Val Leu Val Ile Thr Glu 705 710 715			2163
aaa ggg tac gcc aaa cga acc tcc caa gaa gag tac acc ctc cac aag Lys Gly Tyr Gly Lys Arg Thr Ser Gln Glu Glu Tyr Thr Leu His Lys 720 725 730			2211
cga ggg gcc aag ggg gtt aaa acc ctt cat att acc gat aag aat ggt Arg Gly Gly Lys Gly Val Lys Thr Leu His Ile Thr Asp Lys Asn Gly 735 740 745 750			2259
ccc cta att gga ctg aaa act gtc tct ggt ggt gag gac gtc atg att Pro Leu Ile Gly Leu Lys Thr Val Ser Gly Gly Glu Asp Val Met Ile 755 760 765			2307
gtc acc gac caa ggt atc atg att cgt atc gaa gcc gac agc atc tct Val Thr Asp Gln Gly Ile Met Ile Arg Ile Glu Ala Asp Ser Ile Ser 770 775 780			2355
cag acc tcc cgc cta acc caa ggt gtc cgt tta att cga ctt gaa gaa Gln Thr Ser Arg Leu Thr Gln Gly Val Arg Leu Ile Arg Leu Glu Glu 785 790 795			2403
gat agc cgg gtg tca acg gta gcc ctc att gat att gac caa gag ctt Asp Ser Arg Val Ser Thr Val Ala Leu Ile Asp Ile Asp Gln Glu Leu 800 805 810			2451
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<211> 824

<212> PRT

<213> Alloiococcus otitidis

<400> 18

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20 25 30

Ser Arg Ala Leu Pro Asp Val Arg Asp Gly Leu Lys Pro Val His Arg
35 40 45

Arg Ile Leu Tyr Gly Met Asn Glu Leu Gly Leu Thr Pro Asp Lys Ser
50 55 60

Tyr Lys Lys Ser Ala Arg Ile Val Gly Asp Val Met Gly Lys Tyr His
65 70 75 80

Pro His Gly Asp Thr Ala Ile Tyr Asp Ser Met Val Arg Met Ala Gln
85 90 95

Asp Phe Ser Tyr Arg Val Pro Leu Val Asp Gly His Gly Asn Phe Gly
100 105 110

Ser Val Asp Gly Asp Gly Ala Ala Ala Met Arg Tyr Thr Glu Ala Arg
115 120 125

Met Ser Lys Met Ala Leu Glu Leu Leu Arg Asp Ile Asn Lys Asp Thr
130 135 140

Ile Asp Tyr His Asp Asn Tyr Asp Gly Thr Glu Ser Glu Pro Asp Ile
145 150 155 160

Leu Pro Ala Arg Phe Pro Asn Leu Leu Val Asn Gly Ala Ser Gly Ile
165 170 175

Ala Val Gly Met Ala Thr Asn Ile Pro Pro His Asn Leu Lys Glu Val
180 185 190

Ile Asp Ala Cys Val Leu Leu Met Glu Asn Glu Asp Val Thr Val Ala
195 200 205

Asp Leu Met Glu Val Leu Pro Gly Pro Asp Phe Pro Thr Gly Ala Ser
210 215 220

Leu Ile Gly Val Ser Gly Val Arg Lys Ala Tyr Glu Thr Gly Arg Gly
225 230 235 240

Ser Ile Lys Leu Arg Ala Lys Ser Arg Ile Asp Val Asp Gln Lys Gly

245

250

255

Lys Glu Arg Ile Ile Ile Asp Glu Ile Pro Tyr Met Val Asn Lys Ala
260 265 270

Lys Leu Val Glu Lys Ile Ala Glu Leu Ala Arg Asp Lys Lys Ile Asp
275 280 285

Gly Ile Thr Asp Leu Asn Asp Glu Ser Asp Arg Glu Gly Leu Arg Ile
290 295 300

Val Ile Asp Val Arg Arg Asp Thr Ser Ala Gly Ile Leu Leu Asn Lys
305 310 315 320

Leu Tyr Lys Met Thr Gln Leu Gln Val Ser Phe Gly Phe Asn Met Leu
325 330 335

Ala Ile Val Asp Gly Val Pro Lys Thr Leu Gly Leu Lys Asp Ile Leu
340 345 350

Thr His Tyr Leu Asp His Gln Lys Thr Val Ile Arg Arg Arg Thr Glu
355 360 365

Phe Asp Lys Asn Lys Ala Glu Ser Arg Ala His Ile Leu Glu Gly Leu
370 375 380

Arg Thr Ala Leu Asp His Ile Asp Ala Ile Ile Thr Ile Ile Arg Gln
385 390 395 400

Ser Gln Gln Ala Glu Glu Ala Lys Ser Gln Leu Met Ala Ser Tyr Asp
405 410 415

Leu Ser Asp Arg Gln Ala Gln Ala Ile Leu Asp Met Arg Met Val Arg
420 425 430

Leu Thr Gly Leu Glu Arg Glu Lys Ile Glu Asp Glu Tyr Ala Glu Leu
435 440 445

Leu Glu Lys Ile Glu Asp Leu Arg Asp Ile Leu Ala Arg Pro Glu Arg
450 455 460

Ile Lys Gln Ile Ile Lys Glu Glu Met Ile Glu Ile Ala Glu Lys His
465 470 475 480

Gly Gln Asp Arg Leu Thr Asp Ile Arg Val Gly Glu Glu Leu Ser Ile
485 490 495

Glu Asp Glu Asp Leu Ile Glu Glu Glu Asp Ile Ile Ile Thr Leu Ser
500 505 510

Arg Lys Gly Tyr Ile Lys Arg Met Pro Ala Gly Glu Phe Lys Ala Gln
515 520 525

Asn Arg Gly Gly Arg Gly Val Lys Gly Met Thr Thr Asn Asp Gly Asp
530 535 540

Phe Val Glu Gln Leu Thr Phe Cys Ser Ser His Asp Gln Ile Leu Phe
545 550 555 560

Phe Thr Asn Gln Gly Lys Val Tyr Lys Ile Lys Ala Tyr Glu Ile Pro
565 570 575

Glu Tyr Gly Arg Asn Ala Lys Gly Ile Pro Ala Ile Asn Phe Leu Asn
580 585 590

Ile Asp Lys Asp Glu Tyr Ile Gln Ala Met Val Asn Leu Thr Asp Gln
595 600 605

Ala Asp Asp Gln Asp Gln Phe Phe Phe Ala Thr Arg Leu Gly Arg Val
610 615 620

Lys Arg Thr Ala Gln Ser Glu Phe Gln Asn Ile Arg Ser Ser Gly Leu
625 630 635 640

Asn Ala Ile Asn Leu Asn Glu Gly Asp Glu Leu Val Asn Val Val Pro
645 650 655

Thr His Asn Asp Gln Ala Ile Ile Leu Ala Ser Gln Gln Gly Tyr Ala
660 665 670

Val Tyr Phe Asp Glu Lys Asp Ile Arg Ser Met Gly Arg Gly Ala Ala
675 680 685

Gly Val Arg Gly Ile Arg Leu Gly Asp Gly Asp Thr Val Val Ala Met
690 695 700

Glu Val Leu Glu Pro Gly Gln Asp Val Leu Val Ile Thr Glu Lys Gly
705 710 715 720

Tyr Gly Lys Arg Thr Ser Gln Glu Glu Tyr Thr Leu His Lys Arg Gly
725 730 735

Gly Lys Gly Val Lys Thr Leu His Ile Thr Asp Lys Asn Gly Pro Leu
740 745 750

Ile Gly Leu Lys Thr Val Ser Gly Gly Glu Asp Val Met Ile Val Thr
755 760 765

Asp Gln Gly Ile Met Ile Arg Ile Glu Ala Asp Ser Ile Ser Gln Thr
770 775 780

Ser Arg Leu Thr Gln Gly Val Arg Leu Ile Arg Leu Glu Glu Asp Ser
785 790 795 800

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Gln Val Asn Gln Thr Val Glu Glu
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<211> 1956
<212> DNA
<213> *Alloioococcus otitidis*

<220>
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<222> (7) .. (1956)
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ctg aaa aaa aca tat gat gct agt caa atc caa gtc tta gaa ggc cta 96
Leu Lys Lys Thr Tyr Asp Ala Ser Gln Ile Gln Val Leu Glu Gly Leu
15 20 25 30

gaa gca gtc aga gta cgg ccg ggt atg tac att ggg tcc acc agc aag 144
Glu Ala Val Arg Val Arg Pro Gly Met Tyr Ile Gly Ser Thr Ser Lys
35 40 45

gaa ggc ctc cac cac ttg gta tgg gag atc gtg gac aat gct att gac 192

Glu Gly Leu His His Leu Val Trp Glu Ile Val Asp Asn Ala Ile Asp	
50 55 60	
gaa gct atg gcc ggt tat gcc gac aag att tct gtt tcc att ttg gaa	240
Glu Ala Met Ala Gly Tyr Ala Asp Lys Ile Ser Val Ser Ile Leu Glu	
65 70 75	
ggc gac gtg atc caa gtg gct gat aac ggc cgg ggc atc ccg gtt gat	288
Gly Asp Val Ile Gln Val Ala Asp Asn Gly Arg Gly Ile Pro Val Asp	
80 85 90	
atc cag gaa aaa aca ggc cgg cca gct gtt gaa act gtc ttt aca gtc	336
Ile Gln Glu Lys Thr Gly Arg Pro Ala Val Glu Thr Val Phe Thr Val	
95 100 105 110	
ctc cac gct ggt ggg aaa ttt ggt ggc ggt ggt tac aag gtt tcc ggt	384
Leu His Ala Gly Gly Lys Phe Gly Gly Gly Gly Tyr Lys Val Ser Gly	
115 120 125	
ggt ctg cac ggt gta ggg tct tct gtg gtc aat gct ctc tcc gaa tac	432
Gly Leu His Gly Val Gly Ser Ser Val Val Asn Ala Leu Ser Glu Tyr	
130 135 140	
ctc caa gtc cag gtg cac cga gat ggt aaa atc tac caa caa gtt tac	480
Leu Gln Val Gln Val His Arg Asp Gly Lys Ile Tyr Gln Gln Val Tyr	
145 150 155	
aag cgg ggc ttg gtt gat tct gac ttg gaa gtg gtg ggt gag act gac	528
Lys Arg Gly Leu Val Asp Ser Asp Leu Glu Val Val Gly Glu Thr Asp	
160 165 170	
cac act gga act att gtt acc ttt aag gca gat agt ttg att ttt aaa	576
His Thr Gly Thr Ile Val Thr Phe Lys Ala Asp Ser Leu Ile Phe Lys	
175 180 185 190	
gac act act tct tat gac ttc aat acc tta gcc acc cgg atc cgg gag	624
Asp Thr Thr Ser Tyr Asp Phe Asn Thr Leu Ala Thr Arg Ile Arg Glu	
195 200 205	
ttg gcc ttc tta aac cga ggc ttg aat att tcc atc gaa gac aaa cgg	672
Leu Ala Phe Leu Asn Arg Gly Leu Asn Ile Ser Ile Glu Asp Lys Arg	
210 215 220	
caa gca ggc ggg cag tct ttg aac tac cac tat gaa ggt ggg ata tcg	720
Gln Ala Gly Gly Gln Ser Leu Asn Tyr His Tyr Glu Gly Gly Ile Ser	
225 230 235	
agt tat gtt gac cac ttg aat tcc agc cgt gaa gtt ctt tat gag acc	768
Ser Tyr Val Asp His Leu Asn Ser Ser Arg Glu Val Leu Tyr Glu Thr	
240 245 250	
cca att ttc ttg gaa ggg gaa gaa gaa ggg att tct gtg gaa att gcc	816
Pro Ile Phe Leu Glu Gly Glu Glu Glu Gly Ile Ser Val Glu Ile Ala	
255 260 265 270	
ctc cag cat acc gat agc ttc cat act aat tta atg agt ttt gcc aat	864
Leu Gln His Thr Asp Ser Phe His Thr Asn Leu Met Ser Phe Ala Asn	

275	280	285	
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gcc ctt acc cgg gcg gtc aac gac tat gcc cgg cag aat aac ttg ctc Ala Leu Thr Arg Ala Val Asn Asp Tyr Ala Arg Gln Asn Asn Leu Leu 305 310 315			960
cga gag tca gag gat aac ttt acc ggc gat gac gtt cgg gaa ggt ctg Arg Glu Ser Glu Asp Asn Phe Thr Gly Asp Asp Val Arg Glu Gly Leu 320 325 330			1008
acg gtg gtt ttg tca atc aag cac cca gac ccc caa ttt gaa gga caa Thr Val Val Leu Ser Ile Lys His Pro Asp Pro Gln Phe Glu Gly Gln 335 340 345 350			1056
acc aag act aag ctg ggg aac tct gaa gtc aga ggg ata att gac cgg Thr Lys Thr Lys Leu Gly Asn Ser Glu Val Arg Gly Ile Ile Asp Arg 355 360 365			1104
ctc ttt agc cag cac ttt gaa cgt tac ctc atg gaa aat cca aag gtt Leu Phe Ser Gln His Phe Glu Arg Tyr Leu Met Glu Asn Pro Lys Val 370 375 380			1152
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gca gcc aag aga gcc cgg gaa gtc acc cgg aag aaa tca ggc tta gaa Ala Ala Lys Arg Ala Arg Glu Val Thr Arg Lys Lys Ser Gly Leu Glu 400 405 410			1248
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gaa gaa tcc gaa ctc ttt att gta gaa ggg gat tca gct gga ggg tcg Glu Glu Ser Glu Leu Phe Ile Val Glu Gly Asp Ser Ala Gly Gly Ser 435 440 445			1344
gct aag caa ggt cgg tcc cgg gtt ttc cag gct att ttg ccg att cgt Ala Lys Gln Gly Arg Ser Arg Val Phe Gln Ala Ile Leu Pro Ile Arg 450 455 460			1392
ggc aag att ttg aat gtc gaa aaa gcc agc att gac cgt atc tta gcc Gly Lys Ile Leu Asn Val Glu Lys Ala Ser Ile Asp Arg Ile Leu Ala 465 470 475			1440
aat gaa gaa atc cgg tct ctc ttt aca gcc atg gga act ggc ttc ggg Asn Glu Glu Ile Arg Ser Leu Phe Thr Ala Met Gly Thr Gly Phe Gly 480 485 490			1488
gaa gaa ttt aat gtt gaa gaa gct cgc tac aat aag tta att atc atg Glu Glu Phe Asn Val Glu Glu Ala Arg Tyr Asn Lys Leu Ile Ile Met 495 500 505 510			1536

aca gat gct gat gtt gac gga gcc cac att cgg acc ttg ctc ttg acc 1584
 Thr Asp Ala Asp Val Asp Gly Ala His Ile Arg Thr Leu Leu Leu Thr
 515 520 525
 ctt ctt tac cgg tat atg cgg ccc ttg att gaa gca ggt ttc gtc tac 1632
 Leu Leu Tyr Arg Tyr Met Arg Pro Leu Ile Glu Ala Gly Phe Val Tyr
 530 535 540
 att gcc cag cca ccc ctc tac cag gtc aag caa ggc aag aag gtt aaa 1680
 Ile Ala Gln Pro Pro Leu Tyr Gln Val Lys Gln Gly Lys Lys Val Lys
 545 550 555
 tac ttt gat agt gac cgg gaa ctg gac tcc tac ttg aaa gaa att cct 1728
 Tyr Phe Asp Ser Asp Arg Glu Leu Asp Ser Tyr Leu Lys Glu Ile Pro
 560 565 570
 gac tca ccc aag cct tct gtc caa cgc tac aaa ggc tta gga gaa atg 1776
 Asp Ser Pro Lys Pro Ser Val Gln Arg Tyr Lys Gly Leu Gly Glu Met
 575 580 585 590
 gat gct gag cag ttg tgg gaa acc acc atg aac cca gaa cac cgc cgc 1824
 Asp Ala Glu Gln Leu Trp Glu Thr Thr Met Asn Pro Glu His Arg Arg
 595 600 605
 tta ctt cgg gta gac gta gac gac gcc att gag gct gac act att ttt 1872
 Leu Leu Arg Val Asp Val Asp Asp Ala Ile Glu Ala Asp Thr Ile Phe
 610 615 620
 gac atg ttg atg ggt gag gat gtc aaa ccc cgg cgc gac ttt atc aaa 1920
 Asp Met Leu Met Gly Glu Asp Val Lys Pro Arg Arg Asp Phe Ile Lys
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 640 645

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<211> 649

<212> PRT

<213> *Alloioococcus otitidis*

<400> 20

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Val Arg Val Arg Pro Gly Met Tyr Ile Gly Ser Thr Ser Lys Glu Gly
 35 40 45

Leu His His Leu Val Trp Glu Ile Val Asp Asn Ala Ile Asp Glu Ala

50

55

60

Met Ala Gly Tyr Ala Asp Lys Ile Ser Val Ser Ile Leu Glu Gly Asp
65 70 75 80

Val Ile Gln Val Ala Asp Asn Gly Arg Gly Ile Pro Val Asp Ile Gln
85 90 95

Glu Lys Thr Gly Arg Pro Ala Val Glu Thr Val Phe Thr Val Leu His
100 105 110

Ala Gly Gly Lys Phe Gly Gly Gly Gly Tyr Lys Val Ser Gly Gly Leu
115 120 125

His Gly Val Gly Ser Ser Val Val Asn Ala Leu Ser Glu Tyr Leu Gln
130 135 140

Val Gln Val His Arg Asp Gly Lys Ile Tyr Gln Gln Val Tyr Lys Arg
145 150 155 160

Gly Leu Val Asp Ser Asp Leu Glu Val Val Gly Glu Thr Asp His Thr
165 170 175

Gly Thr Ile Val Thr Phe Lys Ala Asp Ser Leu Ile Phe Lys Asp Thr
180 185 190

Thr Ser Tyr Asp Phe Asn Thr Leu Ala Thr Arg Ile Arg Glu Leu Ala
195 200 205

Phe Leu Asn Arg Gly Leu Asn Ile Ser Ile Glu Asp Lys Arg Gln Ala
210 215 220

Gly Gly Gln Ser Leu Asn Tyr His Tyr Glu Gly Gly Ile Ser Ser Tyr
225 230 235 240

Val Asp His Leu Asn Ser Ser Arg Glu Val Leu Tyr Glu Thr Pro Ile
245 250 255

Phe Leu Glu Gly Glu Glu Gly Ile Ser Val Glu Ile Ala Leu Gln
260 265 270

His Thr Asp Ser Phe His Thr Asn Leu Met Ser Phe Ala Asn Asn Ile
275 280 285

His Thr Tyr Glu Gly Gly Met His Ile Ser Gly Phe Lys Thr Ala Leu
290 295 300

Thr Arg Ala Val Asn Asp Tyr Ala Arg Gln Asn Asn Leu Leu Arg Glu
305 310 315 320

Ser Glu Asp Asn Phe Thr Gly Asp Asp Val Arg Glu Gly Leu Thr Val
325 330 335

Val Leu Ser Ile Lys His Pro Asp Pro Gln Phe Glu Gly Gln Thr Lys
340 345 350

Thr Lys Leu Gly Asn Ser Glu Val Arg Gly Ile Ile Asp Arg Leu Phe
355 360 365

Ser Gln His Phe Glu Arg Tyr Leu Met Glu Asn Pro Lys Val Gly Lys
370 375 380

Arg Ile Val Asp Lys Ala Leu Leu Ala Ser Lys Ala Arg Gln Ala Ala
385 390 395 400

Lys Arg Ala Arg Glu Val Thr Arg Lys Lys Ser Gly Leu Glu Ile Ser
405 410 415

Asn Leu Pro Gly Lys Leu Ala Asp Cys Ser Ser Lys Asp Pro Glu Glu
420 425 430

Ser Glu Leu Phe Ile Val Glu Gly Asp Ser Ala Gly Gly Ser Ala Lys
435 440 445

Gln Gly Arg Ser Arg Val Phe Gln Ala Ile Leu Pro Ile Arg Gly Lys
450 455 460

Ile Leu Asn Val Glu Lys Ala Ser Ile Asp Arg Ile Leu Ala Asn Glu
465 470 475 480

Glu Ile Arg Ser Leu Phe Thr Ala Met Gly Thr Gly Phe Gly Glu Glu
485 490 495

Phe Asn Val Glu Glu Ala Arg Tyr Asn Lys Leu Ile Ile Met Thr Asp
500 505 510

Ala Asp Val Asp Gly Ala His Ile Arg Thr Leu Leu Leu Thr Leu Leu
515 520 525

Tyr Arg Tyr Met Arg Pro Leu Ile Glu Ala Gly Phe Val Tyr Ile Ala
530 535 540

Gln Pro Pro Leu Tyr Gln Val Lys Gln Gly Lys Lys Val Lys Tyr Phe
545 550 555 560

Asp Ser Asp Arg Glu Leu Asp Ser Tyr Leu Lys Glu Ile Pro Asp Ser
565 570 575

Pro Lys Pro Ser Val Gln Arg Tyr Lys Gly Leu Gly Glu Met Asp Ala
580 585 590

Glu Gln Leu Trp Glu Thr Thr Met Asn Pro Glu His Arg Arg Leu Leu
595 600 605

Arg Val Asp Val Asp Asp Ala Ile Glu Ala Asp Thr Ile Phe Asp Met
610 615 620

Leu Met Gly Glu Asp Val Lys Pro Arg Arg Asp Phe Ile Lys Glu Asn
625 630 635 640

Ala Arg Tyr Val Glu Asn Ile Asp Ile
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<211> 1218
<212> DNA
<213> *Alloiococcus otitidis*

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<222> (16)..(1218)
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Leu Lys Asn Lys Arg Lys Phe Gly Gly Ile Phe Leu Lys Phe Ser Val
15 20 25

aaa cgg acg gaa ttt cta aaa gta tta aaa aaa gta cag att gca gtg 147

Lys	Arg	Thr	Glu	Phe	Leu	Lys	Val	Leu	Lys	Lys	Val	Gln	Ile	Ala	Val		
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tct	tct	aaa	agt	acc	atc	gct	atc	ttg	acc	ggg	att	aaa	tta	gaa	gcg	195	
Ser	Ser	Lys	Ser	Thr	Ile	Ala	Ile	Leu	Thr	Gly	Ile	Lys	Leu	Glu	Ala		
45					50					55				60			
gat	aac	cag	ggt	tta	acc	tta	acc	gga	tct	aac	tcg	gat	atc	tca	ggt	243	
Asp	Asn	Gln	Gly	Leu	Thr	Leu	Thr	Gly	Ser	Asn	Ser	Asp	Ile	Ser	Val		
				65				70						75			
gaa	agt	tac	tta	tct	gtg	acc	gat	gaa	ggg	gcg	gat	ttg	ggt	att	gat	291	
Glu	Ser	Tyr	Leu	Ser	Val	Thr	Asp	Glu	Gly	Ala	Asp	Leu	Val	Ile	Asp		
			80					85					90				
gag	ccg	ggg	cag	att	gtc	ttg	caa	cca	gcc	cgg	tta	ttt	gcc	aat	atc	339	
Glu	Pro	Gly	Gln	Ile	Val	Leu	Gln	Pro	Ala	Arg	Leu	Phe	Ala	Asn	Ile		
		95					100					105					
gtc	caa	aaa	tta	ccg	gac	acc	cac	ttt	aag	gta	aac	ggt	agc	caa	ggc	387	
Val	Gln	Lys	Leu	Pro	Asp	Thr	His	Phe	Lys	Val	Asn	Val	Ser	Gln	Gly		
		110				115					120						
cag	caa	acc	caa	atc	acc	tca	gct	tca	gcc	tcc	ttt	act	atc	aac	ggc	435	
Gln	Gln	Thr	Gln	Ile	Thr	Ser	Ala	Ser	Ala	Ser	Phe	Thr	Ile	Asn	Gly		
125					130					135					140		
att	gac	gcc	atg	tcc	tac	ccc	cac	ttg	cca	gat	atc	gac	ctg	gag	gaa	483	
Ile	Asp	Ala	Met	Ser	Tyr	Pro	His	Leu	Pro	Asp	Ile	Asp	Leu	Glu	Glu		
				145					150					155			
tcc	ttt	acc	ctg	ccg	ggt	gac	ctc	ttt	aaa	aac	atg	atc	aac	cag	act	531	
Ser	Phe	Thr	Leu	Pro	Val	Asp	Leu	Phe	Lys	Asn	Met	Ile	Asn	Gln	Thr		
			160					165					170				
gtc	atc	gca	gtc	tcc	aac	cat	gaa	agt	cgg	ccc	atc	cta	act	ggg	ggt	579	
Val	Ile	Ala	Val	Ser	Asn	His	Glu	Ser	Arg	Pro	Ile	Leu	Thr	Gly	Val		
		175					180					185					
aac	cta	tct	ctc	aaa	gag	ggc	cga	ctc	aag	gca	gtg	gca	acc	gac	agc	627	
Asn	Leu	Ser	Leu	Lys	Glu	Gly	Arg	Leu	Lys	Ala	Val	Ala	Thr	Asp	Ser		
		190				195					200						
cac	cgt	ttg	tcg	caa	cgg	tcc	atc	caa	tta	gag	tca	gcg	cct	gat	att	675	
His	Arg	Leu	Ser	Gln	Arg	Ser	Ile	Gln	Leu	Glu	Ser	Ala	Pro	Asp	Ile		
205					210					215				220			
tcc	ttt	gac	att	gtg	ata	cca	ggt	aag	tct	ttg	act	gaa	ctg	act	aag	723	
Ser	Phe	Asp	Ile	Val	Ile	Pro	Gly	Lys	Ser	Leu	Thr	Glu	Leu	Thr	Lys		
				225					230					235			
ttg	atg	gat	gca	gat	gaa	gaa	gtc	cgg	gta	gcc	atc	agc	gac	aac	caa	771	
Leu	Met	Asp	Ala	Asp	Glu	Glu	Val	Arg	Val	Ala	Ile	Ser	Asp	Asn	Gln		
			240				245						250				
atc	cta	ttt	gcc	ctc	tcc	agc	agc	cag	ttt	tac	tct	cgg	ctc	cta	gaa	819	
Ile	Leu	Phe	Ala	Leu	Ser	Ser	Ser	Gln	Phe	Tyr	Ser	Arg	Leu	Leu	Glu		

255	260	265	
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tcc ctc ctc tcc cat gaa ggg aaa aac aat gtg gtc caa ctc aca gtg Ser Leu Leu Ser His Glu Gly Lys Asn Asn Val Val Gln Leu Thr Val 305 310 315			963
act gct gaa aag ttg gaa atc gaa ggc cag tca gct gaa gtg ggc cat Thr Ala Glu Lys Leu Glu Ile Glu Gly Gln Ser Ala Glu Val Gly His 320 325 330			1011
gtc caa gaa gaa att gac ttt ggc cac ttc caa ggc caa gac tta acc Val Gln Glu Glu Ile Asp Phe Gly His Phe Gln Gly Gln Asp Leu Thr 335 340 345			1059
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caa gga gaa att aag ttg aaa tta gtt tcg acc ttg cga ccc ttt gtc Gln Gly Glu Ile Lys Leu Lys Leu Val Ser Thr Leu Arg Pro Phe Val 365 370 375 380			1155
atc gtc cca agt gag gac caa gga gac ttt atc caa ctt att act cca Ile Val Pro Ser Glu Asp Gln Gly Asp Phe Ile Gln Leu Ile Thr Pro 385 390 395			1203
atc cga aca gcc taa Ile Arg Thr Ala 400			1218

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<211> 400

<212> PRT

<213> Alloiococcus otitidis

<400> 22

Met	Lys	Trp	Arg	Lys	Thr	Lys	Thr	Ile	Tyr	Gly	Ile	Leu	Lys	Asn	Lys
1				5				10						15	

Arg	Lys	Phe	Gly	Gly	Ile	Phe	Leu	Lys	Phe	Ser	Val	Lys	Arg	Thr	Glu
			20					25					30		

Phe	Leu	Lys	Val	Leu	Lys	Lys	Val	Gln	Ile	Ala	Val	Ser	Ser	Lys	Ser
			35				40					45			

Thr Ile Ala Ile Leu Thr Gly Ile Lys Leu Glu Ala Asp Asn Gln Gly
50 55 60

Leu Thr Leu Thr Gly Ser Asn Ser Asp Ile Ser Val Glu Ser Tyr Leu
65 70 75 80

Ser Val Thr Asp Glu Gly Ala Asp Leu Val Ile Asp Glu Pro Gly Gln
85 90 95

Ile Val Leu Gln Pro Ala Arg Leu Phe Ala Asn Ile Val Gln Lys Leu
100 105 110

Pro Asp Thr His Phe Lys Val Asn Val Ser Gln Gly Gln Gln Thr Gln
115 120 125

Ile Thr Ser Ala Ser Ala Ser Phe Thr Ile Asn Gly Ile Asp Ala Met
130 135 140

Ser Tyr Pro His Leu Pro Asp Ile Asp Leu Glu Glu Ser Phe Thr Leu
145 150 155 160

Pro Val Asp Leu Phe Lys Asn Met Ile Asn Gln Thr Val Ile Ala Val
165 170 175

Ser Asn His Glu Ser Arg Pro Ile Leu Thr Gly Val Asn Leu Ser Leu
180 185 190

Lys Glu Gly Arg Leu Lys Ala Val Ala Thr Asp Ser His Arg Leu Ser
195 200 205

Gln Arg Ser Ile Gln Leu Glu Ser Ala Pro Asp Ile Ser Phe Asp Ile
210 215 220

Val Ile Pro Gly Lys Ser Leu Thr Glu Leu Thr Lys Leu Met Asp Ala
225 230 235 240

Asp Glu Glu Val Arg Val Ala Ile Ser Asp Asn Gln Ile Leu Phe Ala
245 250 255

Leu Ser Ser Ser Gln Phe Tyr Ser Arg Leu Leu Glu Gly Lys Tyr Pro
260 265 270

Asp Thr Asp Arg Leu Ile Pro Gly Asp Thr Pro Thr Glu Ile Thr Leu

275

280

285

Asp Thr Lys Glu Leu Gln Gly Ala Val Asp Arg Ala Ser Leu Leu Ser
 290 295 300

His Glu Gly Lys Asn Asn Val Val Gln Leu Thr Val Thr Ala Glu Lys
 305 310 315 320

Leu Glu Ile Glu Gly Gln Ser Ala Glu Val Gly His Val Gln Glu Glu
 325 330 335

Ile Asp Phe Gly His Phe Gln Gly Gln Asp Leu Thr Ile Ser Phe Asn
 340 345 350

Pro Asp Tyr Leu Lys Glu Ala Leu Ala Thr Phe Gly Gln Gly Glu Ile
 355 360 365

Lys Leu Lys Leu Val Ser Thr Leu Arg Pro Phe Val Ile Val Pro Ser
 370 375 380

Glu Asp Gln Gly Asp Phe Ile Gln Leu Ile Thr Pro Ile Arg Thr Ala
 385 390 395 400

<210> 23

<211> 1317

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (25)..(1317)

<223>

<400> 23

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 Met Gln Met Asn Trp Lys Glu Thr Ile
 1 5

agt ctc atc aac acc acc cgg ggg acc gga gac aag aaa aat ttg aac 99
 Ser Leu Ile Asn Thr Thr Arg Gly Thr Gly Asp Lys Lys Asn Leu Asn
 10 15 20 25

cgg atg cga ctt tta ctc aaa gag cta ggt aat cct gaa aca gac ttg 147
 Arg Met Arg Leu Leu Leu Lys Glu Leu Gly Asn Pro Glu Thr Asp Leu
 30 35 40

ccg gtc atc cac gtt gct ggc acc aat ggc aaa ggg acg acc tgt gct Pro Val Ile His Val Ala Gly Thr Asn Gly Lys Gly Thr Thr Cys Ala 45 50 55	195
tat att gcc cac agc ttg gcc cgt gct ggt tat aaa aca gga ctt tac Tyr Ile Ala His Ser Leu Ala Arg Ala Gly Tyr Lys Thr Gly Leu Tyr 60 65 70	243
acc agc ccc cac ctg gag cgg gtc aat gaa cgg atc cgg att aat gac Thr Ser Pro His Leu Glu Arg Val Asn Glu Arg Ile Arg Ile Asn Asp 75 80 85	291
cgc tac ata tcc gac caa gac tta atg gct ttg acc ggt caa att gcc Arg Tyr Ile Ser Asp Gln Asp Leu Met Ala Leu Thr Gly Gln Ile Ala 90 95 100 105	339
ccc atc att gac cat cta gaa gac tgc ttg ggt gag aaa tac tat tct Pro Ile Ile Asp His Leu Glu Asp Cys Leu Gly Glu Lys Tyr Tyr Ser 110 115 120	387
ttt gaa att tta act gcc ctt gcc ttc ttg tac ttc cag caa gca ggg Phe Glu Ile Leu Thr Ala Leu Ala Phe Leu Tyr Phe Gln Gln Ala Gly 125 130 135	435
gtg gac ttt tta gtt tta gaa act ggg gta ggg gga aaa att gat gcg Val Asp Phe Leu Val Leu Glu Thr Gly Val Gly Gly Lys Ile Asp Ala 140 145 150	483
acc aat gtg gtg ccc gct cca ctg gtc tca gtc att atc tct att ggc Thr Asn Val Val Pro Ala Pro Leu Val Ser Val Ile Ile Ser Ile Gly 155 160 165	531
tat gac cac acc cat gtc ttg ggt aat acc ctg gaa gac att acc cgg Tyr Asp His Thr His Val Leu Gly Asn Thr Leu Glu Asp Ile Thr Arg 170 175 180 185	579
cac aag gca ggg att att aag aaa ggc tgt ccg gtg gtg gtg ggc cct His Lys Ala Gly Ile Ile Lys Lys Gly Cys Pro Val Val Val Gly Pro 190 195 200	627
ctt gcc gac cat tta ttg gct att gtt aaa gag gtg tcc aaa gaa atg Leu Ala Asp His Leu Leu Ala Ile Val Lys Glu Val Ser Lys Glu Met 205 210 215	675
gac agt aat tta acc att gtc cat ccc gac aag ttt gac att gtt cat Asp Ser Asn Leu Thr Ile Val His Pro Asp Lys Phe Asp Ile Val His 220 225 230	723
caa acc ctt gac tac cag tcc ttt aaa tac ggt ggg gac ttg gtt tta Gln Thr Leu Asp Tyr Gln Ser Phe Lys Tyr Gly Gly Asp Leu Val Leu 235 240 245	771
gag act caa atg att ggt aac cac cag ctg gta aac act gcc cta gct Glu Thr Gln Met Ile Gly Asn His Gln Leu Val Asn Thr Ala Leu Ala 250 255 260 265	819
tat gaa gcc ttg aag att gtc caa caa tct tac ccc gat ttg aca gat	867

Tyr Glu Ala Leu Lys Ile Val Gln Gln Ser Tyr Pro Asp Leu Thr Asp
 270 275 280
 tta gat ata tta gaa ggc ttg aag acg acc cac tgg cca ggc cgg atg 915
 Leu Asp Ile Leu Glu Gly Leu Lys Thr Thr His Trp Pro Gly Arg Met
 285 290 295
 caa aag cta tct gac cag cca gtg gtt gtt ctt gat ggg gcc cac aac 963
 Gln Lys Leu Ser Asp Gln Pro Val Val Val Leu Asp Gly Ala His Asn
 300 305 310
 gaa atc ggg gtc aag gct ctt aga cag tca att gat cac ttt ttc ccc 1011
 Glu Ile Gly Val Lys Ala Leu Arg Gln Ser Ile Asp His Phe Phe Pro
 315 320 325
 ggc aaa aaa atc acc tat ttt gcc gga atg atg gtc gaa aaa gac ttc 1059
 Gly Lys Lys Ile Thr Tyr Phe Ala Gly Met Met Val Glu Lys Asp Phe
 330 335 340 345
 gcc aaa atg ttt gac ctc ctg ggg gaa aca gct gat aaa ttt tac ttg 1107
 Ala Lys Met Phe Asp Leu Leu Gly Glu Thr Ala Asp Lys Phe Tyr Leu
 350 355 360
 att tca ccc gat ttg act cgc ggt ttt gat gtc gac caa gcc gtt caa 1155
 Ile Ser Pro Asp Leu Thr Arg Gly Phe Asp Val Asp Gln Ala Val Gln
 365 370 375
 tct ttg act gac aag ggc tac cag gct tcc agt gtg gct agc ctc caa 1203
 Ser Leu Thr Asp Lys Gly Tyr Gln Ala Ser Ser Val Ala Ser Leu Gln
 380 385 390
 gcc atc tta gac tac ata aac cag caa gca aaa gca gat gaa att atc 1251
 Ala Ile Leu Asp Tyr Ile Asn Gln Gln Ala Lys Ala Asp Glu Ile Ile
 395 400 405
 att atc ttt ggc tcc ctc tac ttg gtt ggc gac ttc cta aaa ctt tac 1299
 Ile Ile Phe Gly Ser Leu Tyr Leu Val Gly Asp Phe Leu Lys Leu Tyr
 410 415 420 425
 cat gaa gca tcc ggt taa 1317
 His Glu Ala Ser Gly
 430

<210> 24

<211> 430

<212> PRT

<213> *Alloiococcus otitidis*

<400> 24

Met Gln Met Asn Trp Lys Glu Thr Ile Ser Leu Ile Asn Thr Thr Arg
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Gly Thr Gly Asp Lys Lys Asn Leu Asn Arg Met Arg Leu Leu Leu Lys
 20 25 30

Glu Leu Gly Asn Pro Glu Thr Asp Leu Pro Val Ile His Val Ala Gly
35 40 45

Thr Asn Gly Lys Gly Thr Thr Cys Ala Tyr Ile Ala His Ser Leu Ala
50 55 60

Arg Ala Gly Tyr Lys Thr Gly Leu Tyr Thr Ser Pro His Leu Glu Arg
65 70 75 80

Val Asn Glu Arg Ile Arg Ile Asn Asp Arg Tyr Ile Ser Asp Gln Asp
85 90 95

Leu Met Ala Leu Thr Gly Gln Ile Ala Pro Ile Ile Asp His Leu Glu
100 105 110

Asp Cys Leu Gly Glu Lys Tyr Tyr Ser Phe Glu Ile Leu Thr Ala Leu
115 120 125

Ala Phe Leu Tyr Phe Gln Gln Ala Gly Val Asp Phe Leu Val Leu Glu
130 135 140

Thr Gly Val Gly Gly Lys Ile Asp Ala Thr Asn Val Val Pro Ala Pro
145 150 155 160

Leu Val Ser Val Ile Ile Ser Ile Gly Tyr Asp His Thr His Val Leu
165 170 175

Gly Asn Thr Leu Glu Asp Ile Thr Arg His Lys Ala Gly Ile Ile Lys
180 185 190

Lys Gly Cys Pro Val Val Val Gly Pro Leu Ala Asp His Leu Leu Ala
195 200 205

Ile Val Lys Glu Val Ser Lys Glu Met Asp Ser Asn Leu Thr Ile Val
210 215 220

His Pro Asp Lys Phe Asp Ile Val His Gln Thr Leu Asp Tyr Gln Ser
225 230 235 240

Phe Lys Tyr Gly Gly Asp Leu Val Leu Glu Thr Gln Met Ile Gly Asn
245 250 255

His Gln Leu Val Asn Thr Ala Leu Ala Tyr Glu Ala Leu Lys Ile Val
260 265 270

Gln Gln Ser Tyr Pro Asp Leu Thr Asp Leu Asp Ile Leu Glu Gly Leu
275 280 285

Lys Thr Thr His Trp Pro Gly Arg Met Gln Lys Leu Ser Asp Gln Pro
290 295 300

Val Val Val Leu Asp Gly Ala His Asn Glu Ile Gly Val Lys Ala Leu
305 310 315 320

Arg Gln Ser Ile Asp His Phe Phe Pro Gly Lys Lys Ile Thr Tyr Phe
325 330 335

Ala Gly Met Met Val Glu Lys Asp Phe Ala Lys Met Phe Asp Leu Leu
340 345 350

Gly Glu Thr Ala Asp Lys Phe Tyr Leu Ile Ser Pro Asp Leu Thr Arg
355 360 365

Gly Phe Asp Val Asp Gln Ala Val Gln Ser Leu Thr Asp Lys Gly Tyr
370 375 380

Gln Ala Ser Ser Val Ala Ser Leu Gln Ala Ile Leu Asp Tyr Ile Asn
385 390 395 400

Gln Gln Ala Lys Ala Asp Glu Ile Ile Ile Ile Phe Gly Ser Leu Tyr
405 410 415

Leu Val Gly Asp Phe Leu Lys Leu Tyr His Glu Ala Ser Gly
420 425 430

<210> 25

<211> 1653

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (91)..(1653)

<223>

<400> 25

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acttttaaag ttttagttag gaggcttagc ttg tac cgt atc tct atg aaa gac	114
Met Tyr Arg Ile Ser Met Lys Asp	
1 5	
ttg cat gcc cta tta gct agc aag cag cag ttg aaa gaa gtg gtc ggt	162
Leu His Ala Leu Leu Ala Ser Lys Gln Gln Leu Lys Glu Val Val Gly	
10 15 20	
ccc gac caa gtt tgg cat tac aat ttg cct caa ggg gaa ttg gcc gac	210
Pro Asp Gln Val Trp His Tyr Asn Leu Pro Gln Gly Glu Leu Ala Asp	
25 30 35 40	
caa gtt ttt gac aaa ctt tcc tac aat tcc caa act gcc tcc tca gac	258
Gln Val Phe Asp Lys Leu Ser Tyr Asn Ser Gln Thr Ala Ser Ser Asp	
45 50 55	
acc ctt ttc ttt tgc aag ggt gct tcc ttt aaa aga gac tac cta gcc	306
Thr Leu Phe Phe Cys Lys Gly Ala Ser Phe Lys Arg Asp Tyr Leu Ala	
60 65 70	
cag gcg gtt gac cag ggt gtc caa gtc tat att tcc gaa aaa ttg tat	354
Gln Ala Val Asp Gln Gly Val Gln Val Tyr Ile Ser Glu Lys Leu Tyr	
75 80 85	
caa ggc ctg gat gct tat gcc atc att gtc cgt gac atc cgc cag acc	402
Gln Gly Leu Asp Ala Tyr Ala Ile Ile Val Arg Asp Ile Arg Gln Thr	
90 95 100	
atg gcc cta gtc gct aag gct ttt tac cag gct cca gat gaa aaa ttg	450
Met Ala Leu Val Ala Lys Ala Phe Tyr Gln Ala Pro Asp Glu Lys Leu	
105 110 115 120	
acc ctg att ggc att acc ggg acc aag ggc aag aca acc aca agt tac	498
Thr Leu Ile Gly Ile Thr Gly Thr Lys Gly Lys Thr Thr Thr Ser Tyr	
125 130 135	
ctc ctc aaa tcc atc ctg gac cag gac caa gcc ggt aag aca gct att	546
Leu Leu Lys Ser Ile Leu Asp Gln Asp Gln Ala Gly Lys Thr Ala Ile	
140 145 150	
att tca acc ttg ggg att tcc tta gac ggc cag acc caa gaa gaa gcc	594
Ile Ser Thr Leu Gly Ile Ser Leu Asp Gly Gln Thr Gln Glu Glu Ala	
155 160 165	
tcc ctg acc act cct gaa gcc ttg gac ctc tac cag atg att gcc cgg	642
Ser Leu Thr Thr Pro Glu Ala Leu Asp Leu Tyr Gln Met Ile Ala Arg	
170 175 180	
gcc caa gac cag ggg atg gac caa ttg att atg gaa gta tct agc caa	690
Ala Gln Asp Gln Gly Met Asp Gln Leu Ile Met Glu Val Ser Ser Gln	
185 190 195 200	
gcc tac aag atg gac cgg gtc tat gga ctg act ttc gac ttt gga gcc	738
Ala Tyr Lys Met Asp Arg Val Tyr Gly Leu Thr Phe Asp Phe Gly Ala	
205 210 215	

ttc tta aat att tcg cct gac cat atc ggc cct aat gag cac cca gat	786
Phe Leu Asn Ile Ser Pro Asp His Ile Gly Pro Asn Glu His Pro Asp	
220 225 230	
atg gaa gat tac ttc tat tgt aaa agt cgt ttg gtt aaa cat tcc aag	834
Met Glu Asp Tyr Phe Tyr Cys Lys Ser Arg Leu Val Lys His Ser Lys	
235 240 245	
ttg gcc ttg ctc aac gct gga ctt gac cag cta gac tac tta aaa gac	882
Leu Ala Leu Leu Asn Ala Gly Leu Asp Gln Leu Asp Tyr Leu Lys Asp	
250 255 260	
ctt agc caa aaa aat ggc ggt cag gtc caa gtt tac ggc caa gat ccc	930
Leu Ser Gln Lys Asn Gly Gly Gln Val Gln Val Tyr Gly Gln Asp Pro	
265 270 275 280	
aag act tgt gac tac tat ttt gag gtt aac aac cag gac agc cgc cgc	978
Lys Thr Cys Asp Tyr Tyr Phe Glu Val Asn Asn Gln Asp Ser Arg Arg	
285 290 295	
ttt gcc att aaa agc caa agc cct gat gac ttg gcc att gat ggg gat	1026
Phe Ala Ile Lys Ser Gln Ser Pro Asp Asp Leu Ala Ile Asp Gly Asp	
300 305 310	
tac caa ttt gaa atg ttg ggt gat ttt aac aag gag aat gcc ctt tgt	1074
Tyr Gln Phe Glu Met Leu Gly Asp Phe Asn Lys Glu Asn Ala Leu Cys	
315 320 325	
gcc gct ctt ata gcg ggg cat tta gaa gtt ggg caa gag gcc att tac	1122
Ala Ala Leu Ile Ala Gly His Leu Glu Val Gly Gln Glu Ala Ile Tyr	
330 335 340	
caa gga ata gcc cag gcc caa gtg cca gga cgg atg cag cat tat act	1170
Gln Gly Ile Ala Gln Ala Gln Val Pro Gly Arg Met Gln His Tyr Thr	
345 350 355 360	
tat ggc aac aat cac atc tat gta gac ttt gcc cac aat tac atc agc	1218
Tyr Gly Asn Asn His Ile Tyr Val Asp Phe Ala His Asn Tyr Ile Ser	
365 370 375	
ttg aaa aat ctt ttt gat ttt gcc caa gac caa cac ccg gac cac acc	1266
Leu Lys Asn Leu Phe Asp Phe Ala Gln Asp Gln His Pro Asp His Thr	
380 385 390	
atg gtg gtt gtc ttg ggg gcc cct ggc aac aag ggg gtg tct cgc cgc	1314
Met Val Val Val Leu Gly Ala Pro Gly Asn Lys Gly Val Ser Arg Arg	
395 400 405	
aag gat atg gga tac ttg ctg tcc caa tac caa ggg gaa gtt atc ttg	1362
Lys Asp Met Gly Tyr Leu Leu Ser Gln Tyr Gln Gly Glu Val Ile Leu	
410 415 420	
acc gaa gat gac ccc aat ttt gaa gac gtt caa gct atc tgc caa gaa	1410
Thr Glu Asp Asp Pro Asn Phe Glu Asp Val Gln Ala Ile Cys Gln Glu	
425 430 435 440	

att gcc caa tac att gat ggc ccc atc cag gtg acc ttt aat gat aac 1458
 Ile Ala Gln Tyr Ile Asp Gly Pro Ile Gln Val Thr Phe Asn Asp Asn
 445 450 455
 cgg ata aat gcc atc caa gac ctc cta gag tcc tta acc cca gaa agt 1506
 Arg Ile Asn Ala Ile Gln Asp Leu Leu Glu Ser Leu Thr Pro Glu Ser
 460 465 470
 caa aaa gtc atc ctg ctt gca ggc aag ggg tcc gac cag tac atg ctg 1554
 Gln Lys Val Ile Leu Leu Ala Gly Lys Gly Ser Asp Gln Tyr Met Leu
 475 480 485
 cgg cgg ggt gtg aag gaa gat tat gcg gga gac cac aaa ttg gtt gaa 1602
 Arg Arg Gly Val Lys Glu Asp Tyr Ala Gly Asp His Lys Leu Val Glu
 490 495 500
 gca ttt tta aac cag caa aag act tct tct cat gag aag ctt gag ggt 1650
 Ala Phe Leu Asn Gln Gln Lys Thr Ser Ser His Glu Lys Leu Glu Gly
 505 510 515 520
 tag 1653

<210> 26
 <211> 520
 <212> PRT
 <213> *Alloiococcus otitidis*

<400> 26
 Met Tyr Arg Ile Ser Met Lys Asp Leu His Ala Leu Leu Ala Ser Lys
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Gln Gln Leu Lys Glu Val Val Gly Pro Asp Gln Val Trp His Tyr Asn
 20 25 30

Leu Pro Gln Gly Glu Leu Ala Asp Gln Val Phe Asp Lys Leu Ser Tyr
 35 40 45

Asn Ser Gln Thr Ala Ser Ser Asp Thr Leu Phe Phe Cys Lys Gly Ala
 50 55 60

Ser Phe Lys Arg Asp Tyr Leu Ala Gln Ala Val Asp Gln Gly Val Gln
 65 70 75 80

Val Tyr Ile Ser Glu Lys Leu Tyr Gln Gly Leu Asp Ala Tyr Ala Ile
 85 90 95

Ile Val Arg Asp Ile Arg Gln Thr Met Ala Leu Val Ala Lys Ala Phe
 100 105 110

Tyr Gln Ala Pro Asp Glu Lys Leu Thr Leu Ile Gly Ile Thr Gly Thr
115 120 125

Lys Gly Lys Thr Thr Thr Ser Tyr Leu Leu Lys Ser Ile Leu Asp Gln
130 135 140

Asp Gln Ala Gly Lys Thr Ala Ile Ile Ser Thr Leu Gly Ile Ser Leu
145 150 155 160

Asp Gly Gln Thr Gln Glu Glu Ala Ser Leu Thr Thr Pro Glu Ala Leu
165 170 175

Asp Leu Tyr Gln Met Ile Ala Arg Ala Gln Asp Gln Gly Met Asp Gln
180 185 190

Leu Ile Met Glu Val Ser Ser Gln Ala Tyr Lys Met Asp Arg Val Tyr
195 200 205

Gly Leu Thr Phe Asp Phe Gly Ala Phe Leu Asn Ile Ser Pro Asp His
210 215 220

Ile Gly Pro Asn Glu His Pro Asp Met Glu Asp Tyr Phe Tyr Cys Lys
225 230 235 240

Ser Arg Leu Val Lys His Ser Lys Leu Ala Leu Leu Asn Ala Gly Leu
245 250 255

Asp Gln Leu Asp Tyr Leu Lys Asp Leu Ser Gln Lys Asn Gly Gly Gln
260 265 270

Val Gln Val Tyr Gly Gln Asp Pro Lys Thr Cys Asp Tyr Tyr Phe Glu
275 280 285

Val Asn Asn Gln Asp Ser Arg Arg Phe Ala Ile Lys Ser Gln Ser Pro
290 295 300

Asp Asp Leu Ala Ile Asp Gly Asp Tyr Gln Phe Glu Met Leu Gly Asp
305 310 315 320

Phe Asn Lys Glu Asn Ala Leu Cys Ala Ala Leu Ile Ala Gly His Leu
325 330 335

Glu Val Gly Gln Glu Ala Ile Tyr Gln Gly Ile Ala Gln Ala Gln Val

340

345

350

Pro Gly Arg Met Gln His Tyr Thr Tyr Gly Asn Asn His Ile Tyr Val
355 360 365

Asp Phe Ala His Asn Tyr Ile Ser Leu Lys Asn Leu Phe Asp Phe Ala
370 375 380

Gln Asp Gln His Pro Asp His Thr Met Val Val Val Leu Gly Ala Pro
385 390 395 400

Gly Asn Lys Gly Val Ser Arg Arg Lys Asp Met Gly Tyr Leu Leu Ser
405 410 415

Gln Tyr Gln Gly Glu Val Ile Leu Thr Glu Asp Asp Pro Asn Phe Glu
420 425 430

Asp Val Gln Ala Ile Cys Gln Glu Ile Ala Gln Tyr Ile Asp Gly Pro
435 440 445

Ile Gln Val Thr Phe Asn Asp Asn Arg Ile Asn Ala Ile Gln Asp Leu
450 455 460

Leu Glu Ser Leu Thr Pro Glu Ser Gln Lys Val Ile Leu Leu Ala Gly
465 470 475 480

Lys Gly Ser Asp Gln Tyr Met Leu Arg Arg Gly Val Lys Glu Asp Tyr
485 490 495

Ala Gly Asp His Lys Leu Val Glu Ala Phe Leu Asn Gln Gln Lys Thr
500 505 510

Ser Ser His Glu Lys Leu Glu Gly
515 520

<210> 27

<211> 636

<212> DNA

<213> Alloiococcus otitidis

<220>

<221> CDS

<222> (25) .. (636)

<223>

<400> 27

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gtt gtt ggg ata ggc ttt ctg att gcc ttt ggc tac acg att tat gac	99
Val Val Gly Ile Gly Phe Leu Ile Ala Phe Gly Tyr Thr Ile Tyr Asp	
10 15 20 25	
cat gct aac tcg aca tcg gtt acc cta gaa gaa gcc cag gtg gcc ctg	147
His Ala Asn Ser Thr Ser Val Thr Leu Glu Glu Ala Gln Val Ala Leu	
30 35 40	
gaa gaa agc cgg gcc cag gct gct gaa gct ggg gac ggg gac cag gat	195
Glu Glu Ser Arg Ala Gln Ala Ala Glu Ala Gly Asp Gly Asp Gln Asp	
45 50 55	
ggc caa gat ggg gcg agt gac atc gat atc caa aac tac cag cct gaa	243
Gly Gln Asp Gly Ala Ser Asp Ile Asp Ile Gln Asn Tyr Gln Pro Glu	
60 65 70	
gct ggg gag gct ttt ggg gtc tta gat att ccc aaa ctc gac cgg agc	291
Ala Gly Glu Ala Phe Gly Val Leu Asp Ile Pro Lys Leu Asp Arg Ser	
75 80 85	
att ggc att gta gcc gga acg gat gca gac tct ctt aag aag ggg gta	339
Ile Gly Ile Val Ala Gly Thr Asp Ala Asp Ser Leu Lys Lys Gly Val	
90 95 100 105	
ggt cac gtt gag aat aca gtc ttc cct ggc caa ggc gaa caa att gtc	387
Gly His Val Glu Asn Thr Val Phe Pro Gly Gln Gly Glu Gln Ile Val	
110 115 120	
ctc tct ggc cac cgg gat acc gtc ttc cgg gac ttt ggc gaa tta gaa	435
Leu Ser Gly His Arg Asp Thr Val Phe Arg Asp Phe Gly Glu Leu Glu	
125 130 135	
att ggc gac aat ttt atc gtt caa atg cct tac ggg gac tat gaa tat	483
Ile Gly Asp Asn Phe Ile Val Gln Met Pro Tyr Gly Asp Tyr Glu Tyr	
140 145 150	
gag att cag gac tat gaa att gtc gac cgg gat gat acc tcc gtc atc	531
Glu Ile Gln Asp Tyr Glu Ile Val Asp Arg Asp Asp Thr Ser Val Ile	
155 160 165	
cgg cct atg ggg gaa gaa gtc tta gtg gtt tca acc tgc tac ccc ttt	579
Arg Pro Met Gly Glu Glu Val Leu Val Val Ser Thr Cys Tyr Pro Phe	
170 175 180 185	
gaa ttt tac ggt ttt gcc cct gac cgc ttt gtt ttc tat tgt tac ccc	627
Glu Phe Tyr Gly Phe Ala Pro Asp Arg Phe Val Phe Tyr Cys Tyr Pro	
190 195 200	
gtt gaa taa	636
Val Glu	

<210> 28

<211> 203

<212> PRT

<213> Alloiococcus otitidis

<400> 28

Met Lys Trp Leu Ser Arg Ile Leu Ile Val Val Gly Ile Gly Phe Leu
1 5 10 15

Ile Ala Phe Gly Tyr Thr Ile Tyr Asp His Ala Asn Ser Thr Ser Val
20 25 30

Thr Leu Glu Glu Ala Gln Val Ala Leu Glu Glu Ser Arg Ala Gln Ala
35 40 45

Ala Glu Ala Gly Asp Gly Asp Gln Asp Gly Gln Asp Gly Ala Ser Asp
50 55 60

Ile Asp Ile Gln Asn Tyr Gln Pro Glu Ala Gly Glu Ala Phe Gly Val
65 70 75 80

Leu Asp Ile Pro Lys Leu Asp Arg Ser Ile Gly Ile Val Ala Gly Thr
85 90 95

Asp Ala Asp Ser Leu Lys Lys Gly Val Gly His Val Glu Asn Thr Val
100 105 110

Phe Pro Gly Gln Gly Glu Gln Ile Val Leu Ser Gly His Arg Asp Thr
115 120 125

Val Phe Arg Asp Phe Gly Glu Leu Glu Ile Gly Asp Asn Phe Ile Val
130 135 140

Gln Met Pro Tyr Gly Asp Tyr Glu Tyr Glu Ile Gln Asp Tyr Glu Ile
145 150 155 160

Val Asp Arg Asp Asp Thr Ser Val Ile Arg Pro Met Gly Glu Glu Val
165 170 175

Leu Val Val Ser Thr Cys Tyr Pro Phe Glu Phe Tyr Gly Phe Ala Pro
180 185 190

Asp Arg Phe Val Phe Tyr Cys Tyr Pro Val Glu
195 200

<210> 29
 <211> 1290
 <212> DNA
 <213> *Alloiococcus otitidis*

<220>
 <221> CDS
 <222> (1)..(1290)
 <223>

<400> 29
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 1 5 10 15

aag atg gat ttg ggg cta gca acc atg acc cag gtg atg gac tta ttg 96
 Lys Met Asp Leu Gly Leu Ala Thr Met Thr Gln Val Met Asp Leu Leu
 20 25 30

ggc aag ccc caa gac cag gtc ccc atg gtt cat atc gct ggc acc aat 144
 Gly Lys Pro Gln Asp Gln Val Pro Met Val His Ile Ala Gly Thr Asn
 35 40 45

ggc aag ggg tcg gcc gca gcc ttt aca gag cga ata ctc agg gag gct 192
 Gly Lys Gly Ser Ala Ala Phe Thr Glu Arg Ile Leu Arg Glu Ala
 50 55 60

ggc tac aag gtc ggc ttg tat att tcc cct tcc cta gtg gaa ttt aat 240
 Gly Tyr Lys Val Gly Leu Tyr Ile Ser Pro Ser Leu Val Glu Phe Asn
 65 70 75 80

gaa cgg atc caa atc aat ggc caa gcc aca agt gat gat cag ttg ctc 288
 Glu Arg Ile Gln Ile Asn Gly Gln Ala Thr Ser Asp Asp Gln Leu Leu
 85 90 95

aag gca gtc aag acc cta agc cag gcc tta gaa ggc aca tcc ctt tgc 336
 Lys Ala Val Lys Thr Leu Ser Gln Ala Leu Glu Gly Thr Ser Leu Cys
 100 105 110

ctg act gaa ttt gaa ctt ttt act gcc ctg gcc ttt ttg acc ttc cag 384
 Leu Thr Glu Phe Glu Leu Phe Thr Ala Leu Ala Phe Leu Thr Phe Gln
 115 120 125

gac cag gct tgt gat ata gcc gtt gta gag gtc gga tta gga gga cgg 432
 Asp Gln Ala Cys Asp Ile Ala Val Val Glu Val Gly Leu Gly Gly Arg
 130 135 140

tta gat gct acc aat gtg ata agc cgt cct gcc gtc acc gcc att acc 480
 Leu Asp Ala Thr Asn Val Ile Ser Arg Pro Ala Val Thr Ala Ile Thr
 145 150 155 160

aag att ggc atg gac cat acc gct ttt tta ggg gat agc ctg cca gaa 528
 Lys Ile Gly Met Asp His Thr Ala Phe Leu Gly Asp Ser Leu Pro Glu
 165 170 175

ata gcc ggt gag aag gca gcc atc gcc aaa gcc ggc tcg cct atg gtg 576

Ile	Ala	Gly	Glu	Lys	Ala	Ala	Ile	Ala	Lys	Ala	Gly	Ser	Pro	Met	Val	
			180					185					190			
gtc	tat	ccc	cag	ggg	cca	gaa	gtg	act	cgg	gtg	atc	caa	aat	cag	gcg	624
Val	Tyr	Pro	Gln	Gly	Pro	Glu	Val	Thr	Arg	Val	Ile	Gln	Asn	Gln	Ala	
		195					200					205				
gac	cgg	gta	gga	gcc	tct	ctg	acc	cta	att	tct	caa	tcc	gac	ctg	act	672
Asp	Arg	Val	Gly	Ala	Ser	Leu	Thr	Leu	Ile	Ser	Gln	Ser	Asp	Leu	Thr	
	210					215					220					
tat	aac	ctg	act	tcg	gac	ctc	ttg	caa	gac	ttt	gaa	tac	aag	cag	gtt	720
Tyr	Asn	Leu	Thr	Ser	Asp	Leu	Leu	Gln	Asp	Phe	Glu	Tyr	Lys	Gln	Val	
225					230				235						240	
ccc	tac	cgc	att	tca	ctt	tta	gaa	gat	tat	caa	att	tac	aac	gcc	ctg	768
Pro	Tyr	Arg	Ile	Ser	Leu	Leu	Glu	Asp	Tyr	Gln	Ile	Tyr	Asn	Ala	Leu	
			245					250						255		
gta	gca	ctc	gaa	atc	tct	ttt	gcc	tta	cag	gat	gct	ggc	tgg	cag	att	816
Val	Ala	Leu	Glu	Ile	Ser	Phe	Ala	Leu	Gln	Asp	Ala	Gly	Trp	Gln	Ile	
		260					265						270			
agc	cct	aaa	gcc	att	aaa	caa	ggg	ttg	gtt	gag	acc	cgc	tgg	ccc	ggc	864
Ser	Pro	Lys	Ala	Ile	Lys	Gln	Gly	Leu	Val	Glu	Thr	Arg	Trp	Pro	Gly	
	275						280					285				
cgt	ttt	gaa	ctt	atc	gcc	tct	cat	ccg	acc	gtg	atc	gtt	gat	ggg	tct	912
Arg	Phe	Glu	Leu	Ile	Ala	Ser	His	Pro	Thr	Val	Ile	Val	Asp	Gly	Ser	
	290					295					300					
cat	aat	gaa	gac	ggc	ctg	cag	gct	ctc	ttg	gct	aac	cta	gac	cgc	tac	960
His	Asn	Glu	Asp	Gly	Leu	Gln	Ala	Leu	Leu	Ala	Asn	Leu	Asp	Arg	Tyr	
305					310					315					320	
ttt	cca	gaa	caa	aaa	agg	att	ggg	atc	gta	ggc	atg	ttg	gcc	gac	aag	1008
Phe	Pro	Glu	Gln	Lys	Arg	Ile	Gly	Ile	Val	Gly	Met	Leu	Ala	Asp	Lys	
			325					330						335		
gat	gtt	gat	gcc	gcc	cta	gct	cct	tta	acc	aaa	agc	ttt	gac	cgg	ctt	1056
Asp	Val	Asp	Ala	Ala	Leu	Ala	Pro	Leu	Thr	Lys	Ser	Phe	Asp	Arg	Leu	
			340				345						350			
tat	acg	gtg	aca	ccc	gat	tcg	ccg	cgg	ggg	atg	gca	gcc	cct	caa	atg	1104
Tyr	Thr	Val	Thr	Pro	Asp	Ser	Pro	Arg	Gly	Met	Ala	Ala	Pro	Gln	Met	
	355						360					365				
aaa	gaa	aaa	ctg	acc	gaa	atg	gtg	tcg	ccg	tct	act	cgg	gtc	ata	gct	1152
Lys	Glu	Lys	Leu	Thr	Glu	Met	Val	Ser	Pro	Ser	Thr	Arg	Val	Ile	Ala	
	370					375					380					
tgt	gaa	agt	tat	aac	cag	gcc	tta	gac	ctg	gca	ggg	caa	gta	gcc	ggc	1200
Cys	Glu	Ser	Tyr	Asn	Gln	Ala	Leu	Asp	Leu	Ala	Gly	Gln	Val	Ala	Gly	
385					390				395						400	
gga	gat	gac	cta	att	gtc	gtt	ttt	gga	agt	ttt	tat	att	gtt	ggg	aag	1248
Gly	Asp	Asp	Leu	Ile	Val	Val	Phe	Gly	Ser	Phe	Tyr	Ile	Val	Gly	Lys	

405

410

415

ttt aga cag ctg att tta gca aga aga aat ggg gaa gtt taa
 Phe Arg Gln Leu Ile Leu Ala Arg Arg Asn Gly Glu Val
 420 425

1290

<210> 30

<211> 429

<212> PRT

<213> Alloiococcus otitidis

<400> 30

Met Gln Tyr Ala Glu Leu Leu Asp Leu Leu Pro Leu Gln Glu Gln Gly
 1 5 10 15

Lys Met Asp Leu Gly Leu Ala Thr Met Thr Gln Val Met Asp Leu Leu
 20 25 30

Gly Lys Pro Gln Asp Gln Val Pro Met Val His Ile Ala Gly Thr Asn
 35 40 45

Gly Lys Gly Ser Ala Ala Ala Phe Thr Glu Arg Ile Leu Arg Glu Ala
 50 55 60

Gly Tyr Lys Val Gly Leu Tyr Ile Ser Pro Ser Leu Val Glu Phe Asn
 65 70 75 80

Glu Arg Ile Gln Ile Asn Gly Gln Ala Thr Ser Asp Asp Gln Leu Leu
 85 90 95

Lys Ala Val Lys Thr Leu Ser Gln Ala Leu Glu Gly Thr Ser Leu Cys
 100 105 110

Leu Thr Glu Phe Glu Leu Phe Thr Ala Leu Ala Phe Leu Thr Phe Gln
 115 120 125

Asp Gln Ala Cys Asp Ile Ala Val Val Glu Val Gly Leu Gly Gly Arg
 130 135 140

Leu Asp Ala Thr Asn Val Ile Ser Arg Pro Ala Val Thr Ala Ile Thr
 145 150 155 160

Lys Ile Gly Met Asp His Thr Ala Phe Leu Gly Asp Ser Leu Pro Glu
 165 170 175

Ile Ala Gly Glu Lys Ala Ala Ile Ala Lys Ala Gly Ser Pro Met Val
180 185 190

Val Tyr Pro Gln Gly Pro Glu Val Thr Arg Val Ile Gln Asn Gln Ala
195 200 205

Asp Arg Val Gly Ala Ser Leu Thr Leu Ile Ser Gln Ser Asp Leu Thr
210 215 220

Tyr Asn Leu Thr Ser Asp Leu Leu Gln Asp Phe Glu Tyr Lys Gln Val
225 230 235 240

Pro Tyr Arg Ile Ser Leu Leu Glu Asp Tyr Gln Ile Tyr Asn Ala Leu
245 250 255

Val Ala Leu Glu Ile Ser Phe Ala Leu Gln Asp Ala Gly Trp Gln Ile
260 265 270

Ser Pro Lys Ala Ile Lys Gln Gly Leu Val Glu Thr Arg Trp Pro Gly
275 280 285

Arg Phe Glu Leu Ile Ala Ser His Pro Thr Val Ile Val Asp Gly Ser
290 295 300

His Asn Glu Asp Gly Leu Gln Ala Leu Leu Ala Asn Leu Asp Arg Tyr
305 310 315 320

Phe Pro Glu Gln Lys Arg Ile Gly Ile Val Gly Met Leu Ala Asp Lys
325 330 335

Asp Val Asp Ala Ala Leu Ala Pro Leu Thr Lys Ser Phe Asp Arg Leu
340 345 350

Tyr Thr Val Thr Pro Asp Ser Pro Arg Gly Met Ala Ala Pro Gln Met
355 360 365

Lys Glu Lys Leu Thr Glu Met Val Ser Pro Ser Thr Arg Val Ile Ala
370 375 380

Cys Glu Ser Tyr Asn Gln Ala Leu Asp Leu Ala Gly Gln Val Ala Gly
385 390 395 400

Gly Asp Asp Leu Ile Val Val Phe Gly Ser Phe Tyr Ile Val Gly Lys

405

410

415

Phe Arg Gln Leu Ile Leu Ala Arg Arg Asn Gly Glu Val
 420 425

<210> 31

<211> 387

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (16) .. (387)

<223>

<400> 31

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Met Asp Lys Arg Asp Lys Ile Arg Leu Gln Gly Met
 1 5 10

act ttt cac ggc cac cac ggt ttg atg gag gcc gaa acc aag ttg ggt 99

Thr Phe His Gly His His Gly Leu Met Glu Ala Glu Thr Lys Leu Gly
 15 20 25

cag att ttt aaa gtc gac ctt gtc tta gta act gac ctc aag tta gcg 147

Gln Ile Phe Lys Val Asp Leu Val Leu Val Thr Asp Leu Lys Leu Ala
 30 35 40

ggc caa aca gac aag atg ggg cac agt atc cac tac ggg gaa gtt tat 195

Gly Gln Thr Asp Lys Met Gly His Ser Ile His Tyr Gly Glu Val Tyr
 45 50 55 60

gac ctg gtc aag tcc att gtg gaa ggt acc ccc ttt aag ctt ttg gag 243

Asp Leu Val Lys Ser Ile Val Glu Gly Thr Pro Phe Lys Leu Leu Glu
 65 70 75

tcc ttg gcg gaa acc cta gcc caa gaa gtt ctc aag act ttt gac cag 291

Ser Leu Ala Glu Thr Leu Ala Gln Glu Val Leu Lys Thr Phe Asp Gln
 80 85 90

gtt gag gag gtc ttg gtc cgg gtc aac aaa ccc cag gcc ccg att cct 339

Val Glu Glu Val Leu Val Arg Val Asn Lys Pro Gln Ala Pro Ile Pro
 95 100 105

ggc gtc ttt gac aat gta gcg gtg gaa atc acc cgg gcc cgt cac tag 387

Gly Val Phe Asp Asn Val Ala Val Glu Ile Thr Arg Ala Arg His
 110 115 120

<210> 32

<211> 123

<212> PRT

<213> *Alloiococcus otitidis*

<400> 32

Met Asp Lys Arg Asp Lys Ile Arg Leu Gln Gly Met Thr Phe His Gly
1 5 10 15

His His Gly Leu Met Glu Ala Glu Thr Lys Leu Gly Gln Ile Phe Lys
20 25 30

Val Asp Leu Val Leu Val Thr Asp Leu Lys Leu Ala Gly Gln Thr Asp
35 40 45

Lys Met Gly His Ser Ile His Tyr Gly Glu Val Tyr Asp Leu Val Lys
50 55 60

Ser Ile Val Glu Gly Thr Pro Phe Lys Leu Leu Glu Ser Leu Ala Glu
65 70 75 80

Thr Leu Ala Gln Glu Val Leu Lys Thr Phe Asp Gln Val Glu Glu Val
85 90 95

Leu Val Arg Val Asn Lys Pro Gln Ala Pro Ile Pro Gly Val Phe Asp
100 105 110

Asn Val Ala Val Glu Ile Thr Arg Ala Arg His
115 120

<210> 33

<211> 552

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (22) .. (552)

<223>

<400> 33

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Met Lys Gly Val Met Ile Gly Leu Gly Ser
1 5 10

aat atg ggg act aag ttg gct tac tta aac cgg gct ttg gcc aaa ata 99
Asn Met Gly Thr Lys Leu Ala Tyr Leu Asn Arg Ala Leu Ala Lys Ile
15 20 25

aat agc cta gac cag gta gca gtc aag caa gtt tca aag gtt tac cag 147
Asn Ser Leu Asp Gln Val Ala Val Lys Gln Val Ser Lys Val Tyr Gln
30 35 40

act gaa ccg gtg ggc tac aag gac cag gac gat ttt tac aat atg gtt 195
Thr Glu Pro Val Gly Tyr Lys Asp Gln Asp Asp Phe Tyr Asn Met Val

45	50	55	
gct ggc ctt gaa att gaa cca ggc aag acc ccc ttg gac ctc tta gaa Ala Gly Leu Glu Ile Glu Pro Gly Lys Thr Pro Leu Asp Leu Leu Glu 60 65 70			243
gac ttg ctg gcg att gag gca gac ctg gac agg aag cgg acc att aaa Asp Leu Leu Ala Ile Glu Ala Asp Leu Asp Arg Lys Arg Thr Ile Lys 75 80 85 90			291
aat ggc ccc cga acc att gac ttg gat gtc ttg ctg gtg gag ggt caa Asn Gly Pro Arg Thr Ile Asp Leu Asp Val Leu Leu Val Glu Gly Gln 95 100 105			339
gaa att gac cat ccc aag ctc caa gtt ccc cac cca agg ctc cag gac Glu Ile Asp His Pro Lys Leu Gln Val Pro His Pro Arg Leu Gln Asp 110 115 120			387
cgg gcc ttt gtc ttg gtc ccc ttg gct gag ttg gac ccc aac tac ctg Arg Ala Phe Val Leu Val Pro Leu Ala Glu Leu Asp Pro Asn Tyr Leu 125 130 135			435
gtt cct ggc ata gat aag aca gtt gcg gac ttg ttg gct tct tta aac Val Pro Gly Ile Asp Lys Thr Val Ala Asp Leu Leu Ala Ser Leu Asn 140 145 150			483
caa acc gac cta gca ggg gtg gag gct ttg ggt cag ttg acg aac cta Gln Thr Asp Leu Ala Gly Val Glu Ala Leu Gly Gln Leu Thr Asn Leu 155 160 165 170			531
tta gaa gac cgt gag gct tga Leu Glu Asp Arg Glu Ala 175			552

Pro Gly Lys Thr Pro Leu Asp Leu Leu Glu Asp Leu Leu Ala Ile Glu
65 70 75 80

Ala Asp Leu Asp Arg Lys Arg Thr Ile Lys Asn Gly Pro Arg Thr Ile
85 90 95

Asp Leu Asp Val Leu Leu Val Glu Gly Gln Glu Ile Asp His Pro Lys
100 105 110

Leu Gln Val Pro His Pro Arg Leu Gln Asp Arg Ala Phe Val Leu Val
115 120 125

Pro Leu Ala Glu Leu Asp Pro Asn Tyr Leu Val Pro Gly Ile Asp Lys
130 135 140

Thr Val Ala Asp Leu Leu Ala Ser Leu Asn Gln Thr Asp Leu Ala Gly
145 150 155 160

Val Glu Ala Leu Gly Gln Leu Thr Asn Leu Leu Glu Asp Arg Glu Ala
165 170 175

<210> 35

<211> 1242

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (40)..(1242)

<223>

<400> 35

aatcttctta atatcgcttg gcccaagacc gctataata gtg gta agt gat tat 54
Met Val Ser Asp Tyr
1 5

ttt agg agg ttc aat atg caa ata gga att gac aag ctg gct ttt gcg 102
Phe Arg Arg Phe Asn Met Gln Ile Gly Ile Asp Lys Leu Ala Phe Ala
10 15 20

act cca acc agg tac ttg gaa atg gcg agt ctg gcc caa gcc cgg tcc 150
Thr Pro Thr Arg Tyr Leu Glu Met Ala Ser Leu Ala Gln Ala Arg Ser
25 30 35

caa gac cct aat aaa tat atc aag ggg cta ggc caa gaa gcc atg gct 198
Gln Asp Pro Asn Lys Tyr Ile Lys Gly Leu Gly Gln Glu Ala Met Ala
40 45 50

gtc cct gaa gaa agt gat gat gcc gtc agc ttg gcg gct aat gcc ggt Val Pro Glu Glu Ser Asp Asp Ala Val Ser Leu Ala Ala Asn Ala Gly 55 60 65	246
aat tta atc tta agt gaa gaa gac aag gct gct att gac atg gtg ata Asn Leu Ile Leu Ser Glu Glu Asp Lys Ala Ala Ile Asp Met Val Ile 70 75 80 85	294
gtc ggt acc gaa tct ggg gtc gac cag tcc aag tcg gca gcc agc tgg Val Gly Thr Glu Ser Gly Val Asp Gln Ser Lys Ser Ala Ala Ser Trp 90 95 100	342
gtt cat gac ctg ttg ggg atc aac ccc cat gct aga agc ctg gag atc Val His Asp Leu Leu Gly Ile Asn Pro His Ala Arg Ser Leu Glu Ile 105 110 115	390
aag caa gcc tgc tac ggg gct acg gct gga ctc aaa cta gct gtg gcc Lys Gln Ala Cys Tyr Gly Ala Thr Ala Gly Leu Lys Leu Ala Val Ala 120 125 130	438
cac cta gcc tta aac cct gac tcc aag gtt tta gtc atc ggt tca gac His Leu Ala Leu Asn Pro Asp Ser Lys Val Leu Val Ile Gly Ser Asp 135 140 145	486
ata gcc aag tat ggt ttg gaa aca ggg ggc gag ccc act caa gga gct Ile Ala Lys Tyr Gly Leu Glu Thr Gly Gly Glu Pro Thr Gln Gly Ala 150 155 160 165	534
ggg gcg gtc gcc atc tta gtc agc cgt gac cct gca att gct gtg gtc Gly Ala Val Ala Ile Leu Val Ser Arg Asp Pro Ala Ile Ala Val Val 170 175 180	582
aac aat gac agt gcc atg ctg acc aaa aat att gca gac ttt tgg cga Asn Asn Asp Ser Ala Met Leu Thr Lys Asn Ile Ala Asp Phe Trp Arg 185 190 195	630
ccc aac tac agc gat tat gcc cat gta gat ggc aag ttc tcc aac cag Pro Asn Tyr Ser Asp Tyr Ala His Val Asp Gly Lys Phe Ser Asn Gln 200 205 210	678
gca tac ttg tcc aac cta gca gaa gtc tgg cgc cag tat aag atc aaa Ala Tyr Leu Ser Asn Leu Ala Glu Val Trp Arg Gln Tyr Lys Ile Lys 215 220 225	726
aac cag ctg tct gct aag gat ttc aag gcc atg gtc ttc cac agc ccc Asn Gln Leu Ser Ala Lys Asp Phe Lys Ala Met Val Phe His Ser Pro 230 235 240 245	774
tat acc aag atg ggg aaa aag gcc tta ctc aaa cta gga gat tat gaa Tyr Thr Lys Met Gly Lys Lys Ala Leu Leu Lys Leu Gly Asp Tyr Glu 250 255 260	822
gac cag aaa gag att gac cgc ttg ctg gcc tat tac gag cct ggt cgc Asp Gln Lys Glu Ile Asp Arg Leu Leu Ala Tyr Tyr Glu Pro Gly Arg 265 270 275	870

tac tac aat aag cgg gtc ggt aat atc tat act ggg tct ctt tac ttg 918
Tyr Tyr Asn Lys Arg Val Gly Asn Ile Tyr Thr Gly Ser Leu Tyr Leu
280 285 290

agt ttg att tcc ctc tta gac cag gta agt gac ctg gag gct ggc gac 966
Ser Leu Ile Ser Leu Leu Asp Gln Val Ser Asp Leu Glu Ala Gly Asp
295 300 305

cgg att ggc ctc tat tct tat ggg tct ggt gcc gtt gga gag ttc ttt 1014
Arg Ile Gly Leu Tyr Ser Tyr Gly Ser Gly Ala Val Gly Glu Phe Phe
310 315 320 325

agc att cgg ctc cag cca ggt tac aag gaa agc tta cag caa gtt gac 1062
Ser Ile Arg Leu Gln Pro Gly Tyr Lys Glu Ser Leu Gln Gln Val Asp
330 335 340

ttc gac cag gtt gtc aac cag cgt tca gca tta gag atg tac agc tat 1110
Phe Asp Gln Val Val Asn Gln Arg Ser Ala Leu Glu Met Tyr Ser Tyr
345 350 355

cag gac ttg ctg acc ttt agc cta cct caa gac ggc caa act tac act 1158
Gln Asp Leu Leu Thr Phe Ser Leu Pro Gln Asp Gly Gln Thr Tyr Thr
360 365 370

aca gat aaa agt cac cag gtc cca ggc cgt ttt gtc tta gac cgg gtg 1206
Thr Asp Lys Ser His Gln Val Pro Gly Arg Phe Val Leu Asp Arg Val
375 380 385

gcc gac cat atc cgt tac tac cgg cgc ttg gct taa 1242
Ala Asp His Ile Arg Tyr Tyr Arg Arg Leu Ala
390 395 400

<210> 36

<211> 400

<212> PRT

<213> Alloiococcus otitidis

<400> 36

Met Val Ser Asp Tyr Phe Arg Arg Phe Asn Met Gln Ile Gly Ile Asp
1 5 10 15

Lys Leu Ala Phe Ala Thr Pro Thr Arg Tyr Leu Glu Met Ala Ser Leu
20 25 30

Ala Gln Ala Arg Ser Gln Asp Pro Asn Lys Tyr Ile Lys Gly Leu Gly
35 40 45

Gln Glu Ala Met Ala Val Pro Glu Glu Ser Asp Asp Ala Val Ser Leu
50 55 60

Ala Ala Asn Ala Gly Asn Leu Ile Leu Ser Glu Glu Asp Lys Ala Ala
65 70 75 80

Ile Asp Met Val Ile Val Gly Thr Glu Ser Gly Val Asp Gln Ser Lys
85 90 95

Ser Ala Ala Ser Trp Val His Asp Leu Leu Gly Ile Asn Pro His Ala
100 105 110

Arg Ser Leu Glu Ile Lys Gln Ala Cys Tyr Gly Ala Thr Ala Gly Leu
115 120 125

Lys Leu Ala Val Ala His Leu Ala Leu Asn Pro Asp Ser Lys Val Leu
130 135 140

Val Ile Gly Ser Asp Ile Ala Lys Tyr Gly Leu Glu Thr Gly Gly Glu
145 150 155 160

Pro Thr Gln Gly Ala Gly Ala Val Ala Ile Leu Val Ser Arg Asp Pro
165 170 175

Ala Ile Ala Val Val Asn Asn Asp Ser Ala Met Leu Thr Lys Asn Ile
180 185 190

Ala Asp Phe Trp Arg Pro Asn Tyr Ser Asp Tyr Ala His Val Asp Gly
195 200 205

Lys Phe Ser Asn Gln Ala Tyr Leu Ser Asn Leu Ala Glu Val Trp Arg
210 215 220

Gln Tyr Lys Ile Lys Asn Gln Leu Ser Ala Lys Asp Phe Lys Ala Met
225 230 235 240

Val Phe His Ser Pro Tyr Thr Lys Met Gly Lys Lys Ala Leu Leu Lys
245 250 255

Leu Gly Asp Tyr Glu Asp Gln Lys Glu Ile Asp Arg Leu Leu Ala Tyr
260 265 270

Tyr Glu Pro Gly Arg Tyr Tyr Asn Lys Arg Val Gly Asn Ile Tyr Thr
275 280 285

Gly Ser Leu Tyr Leu Ser Leu Ile Ser Leu Leu Asp Gln Val Ser Asp
290 295 300

Leu Glu Ala Gly Asp Arg Ile Gly Leu Tyr Ser Tyr Gly Ser Gly Ala
305 310 315 320

Val Gly Glu Phe Phe Ser Ile Arg Leu Gln Pro Gly Tyr Lys Glu Ser
325 330 335

Leu Gln Gln Val Asp Phe Asp Gln Val Val Asn Gln Arg Ser Ala Leu
340 345 350

Glu Met Tyr Ser Tyr Gln Asp Leu Leu Thr Phe Ser Leu Pro Gln Asp
355 360 365

Gly Gln Thr Tyr Thr Thr Asp Lys Ser His Gln Val Pro Gly Arg Phe
370 375 380

Val Leu Asp Arg Val Ala Asp His Ile Arg Tyr Tyr Arg Arg Leu Ala
385 390 395 400

<210> 37

<211> 1323

<212> DNA

<213> *Alloisococcus otitidis*

<220>

<221> CDS

<222> (31) .. (1323)

<223>

<400> 37

ttctggtata gattaaggaa ggaggagacc atg tta ccc tta ttc aag caa ttt 54
Met Leu Pro Leu Phe Lys Gln Phe
1 5

tac aag caa agc ctc agc cag cgc ctc aaa gct cta gaa aag gcc ggc 102
Tyr Lys Gln Ser Leu Ser Gln Arg Leu Lys Ala Leu Glu Lys Ala Gly
10 15 20

tat ctt gat cct gac cag gcg ggt aaa ctc cag tca ggg gaa ctg ggt 150
Tyr Leu Asp Pro Asp Gln Ala Gly Lys Leu Gln Ser Gly Glu Leu Gly
25 30 35 40

ttg acc cat gaa gcc ggc gac cac atg att gaa aac tac atc ggc tcc 198
Leu Thr His Glu Ala Gly Asp His Met Ile Glu Asn Tyr Ile Gly Ser
45 50 55

tat acc ctc cct ctg gga ctg gcc ctc cac ttt tta ctc gat ggc aag 246
Tyr Thr Leu Pro Leu Gly Leu Ala Leu His Phe Leu Leu Asp Gly Lys

60	65	70	
agc tac cta gtc ccc atg gct att gaa gag ccc tct gtc att gcc gct Ser Tyr Leu Val Pro Met Ala Ile Glu Glu Pro Ser Val Ile Ala Ala 75 80 85			294
gcc agc aac ggt gcc aag atg gta gcc caa agc ggt ggt ttc cat aca Ala Ser Asn Gly Ala Lys Met Val Ala Gln Ser Gly Gly Phe His Thr 90 95 100			342
gtc aag gaa aac cgg ctg atg atc ggt caa gtg gtc ata gcc gga agc Val Lys Glu Asn Arg Leu Met Ile Gly Gln Val Val Ile Ala Gly Ser 105 110 115 120			390
aca aaa cct agc cag gac cgg gga aaa atc ctg agc cac cag caa gac Thr Lys Pro Ser Gln Asp Arg Gly Lys Ile Leu Ser His Gln Gln Asp 125 130 135			438
tta atc gac cta gcc aat gct agc tat ccc tca att ggt aaa aga ggg Leu Ile Asp Leu Ala Asn Ala Ser Tyr Pro Ser Ile Gly Lys Arg Gly 140 145 150			486
ggc ggg gcc cga ggc att caa gtc aaa cag ttt gac tca gac ctg ggc Gly Gly Ala Arg Gly Ile Gln Val Lys Gln Phe Asp Ser Asp Leu Gly 155 160 165			534
cag gat atg gga agc tat ctg gca gtc tac ttg act gtt gac tgc cag Gln Asp Met Gly Ser Tyr Leu Ala Val Tyr Leu Thr Val Asp Cys Gln 170 175 180			582
gaa gcc atg ggg gct aac att atc aac acc atg ctg gaa gcc ctg gct Glu Ala Met Gly Ala Asn Ile Ile Asn Thr Met Leu Glu Ala Leu Ala 185 190 195 200			630
cct gaa att gac cgc cta acc agc ggc cag gtc ttg atg tcc atc tta Pro Glu Ile Asp Arg Leu Thr Ser Gly Gln Val Leu Met Ser Ile Leu 205 210 215			678
tct aac ctg gcc act gaa tcc ctt gtc act gtt tcc tgt caa gta aaa Ser Asn Leu Ala Thr Glu Ser Leu Val Thr Val Ser Cys Gln Val Lys 220 225 230			726
ccc aga ttt tta gtc aaa aat gac atg gca ggg gaa gct gtc cgg gac Pro Arg Phe Leu Val Lys Asn Asp Met Ala Gly Glu Ala Val Arg Asp 235 240 245			774
caa atc atc cag gcc tac cag tat gcc tgc ctg gac ccc tac cgg gca Gln Ile Ile Gln Ala Tyr Gln Tyr Ala Cys Leu Asp Pro Tyr Arg Ala 250 255 260			822
gcc acc cac aac aag ggg atc atg aac ggg gta gac ggc ttg gtc cta Ala Thr His Asn Lys Gly Ile Met Asn Gly Val Asp Gly Leu Val Leu 265 270 275 280			870
gct agt ggg aat gat tgg cgg gca atc gaa gcg ggg gcc cat gct tac Ala Ser Gly Asn Asp Trp Arg Ala Ile Glu Ala Gly Ala His Ala Tyr 285 290 295			918

gct agt ttg acc ggc cac tac cgc ccc ttg tcc aag tgg gaa aag acc 966
 Ala Ser Leu Thr Gly His Tyr Arg Pro Leu Ser Lys Trp Glu Lys Thr
 300 305 310

caa gac gga cag tta aaa ggg acc att acc ctt ccc ttg cca att gcc 1014
 Gln Asp Gly Gln Leu Lys Gly Thr Ile Thr Leu Pro Leu Pro Ile Ala
 315 320 325

aca gtt ggt ggg gct att gcc tcc cac cct gta gcc caa gtt agc cag 1062
 Thr Val Gly Gly Ala Ile Ala Ser His Pro Val Ala Gln Val Ser Gln
 330 335 340

caa atc tta ggc caa cct act gct aag caa tta gcc cgg ctg gtt gca 1110
 Gln Ile Leu Gly Gln Pro Thr Ala Lys Gln Leu Ala Arg Leu Val Ala
 345 350 355 360

gca gtg gga cta gcc cag aac cta tcc gct ctt cgt gcc tta gtc aca 1158
 Ala Val Gly Leu Ala Gln Asn Leu Ser Ala Leu Arg Ala Leu Val Thr
 365 370 375

act ggt att caa caa gga cac atg gcc ctc cag gca agg tct ttg gcc 1206
 Thr Gly Ile Gln Gln Gly His Met Ala Leu Gln Ala Arg Ser Leu Ala
 380 385 390

atg aat gcc ggg gcc cgg gga gac aag atc caa aag ctg gca gac cgc 1254
 Met Asn Ala Gly Ala Arg Gly Asp Lys Ile Gln Lys Leu Ala Asp Arg
 395 400 405

tta att aac caa gac caa atg aac cta gca act gcc cgt gcc ctg ctc 1302
 Leu Ile Asn Gln Asp Gln Met Asn Leu Ala Thr Ala Arg Ala Leu Leu
 410 415 420

aag aac atg gaa gaa gac taa 1323
 Lys Asn Met Glu Glu Asp
 425 430

<210> 38

<211> 430

<212> PRT

<213> *Alloiococcus otitidis*

<400> 38

Met Leu Pro Leu Phe Lys Gln Phe Tyr Lys Gln Ser Leu Ser Gln Arg
 1 5 10 15

Leu Lys Ala Leu Glu Lys Ala Gly Tyr Leu Asp Pro Asp Gln Ala Gly
 20 25 30

Lys Leu Gln Ser Gly Glu Leu Gly Leu Thr His Glu Ala Gly Asp His
 35 40 45

Met Ile Glu Asn Tyr Ile Gly Ser Tyr Thr Leu Pro Leu Gly Leu Ala

50

55

60

Leu His Phe Leu Leu Asp Gly Lys Ser Tyr Leu Val Pro Met Ala Ile
 65 70 75 80

Glu Glu Pro Ser Val Ile Ala Ala Ala Ser Asn Gly Ala Lys Met Val
 85 90 95

Ala Gln Ser Gly Gly Phe His Thr Val Lys Glu Asn Arg Leu Met Ile
 100 105 110

Gly Gln Val Val Ile Ala Gly Ser Thr Lys Pro Ser Gln Asp Arg Gly
 115 120 125

Lys Ile Leu Ser His Gln Gln Asp Leu Ile Asp Leu Ala Asn Ala Ser
 130 135 140

Tyr Pro Ser Ile Gly Lys Arg Gly Gly Gly Ala Arg Gly Ile Gln Val
 145 150 155 160

Lys Gln Phe Asp Ser Asp Leu Gly Gln Asp Met Gly Ser Tyr Leu Ala
 165 170 175

Val Tyr Leu Thr Val Asp Cys Gln Glu Ala Met Gly Ala Asn Ile Ile
 180 185 190

Asn Thr Met Leu Glu Ala Leu Ala Pro Glu Ile Asp Arg Leu Thr Ser
 195 200 205

Gly Gln Val Leu Met Ser Ile Leu Ser Asn Leu Ala Thr Glu Ser Leu
 210 215 220

Val Thr Val Ser Cys Gln Val Lys Pro Arg Phe Leu Val Lys Asn Asp
 225 230 235 240

Met Ala Gly Glu Ala Val Arg Asp Gln Ile Ile Gln Ala Tyr Gln Tyr
 245 250 255

Ala Cys Leu Asp Pro Tyr Arg Ala Ala Thr His Asn Lys Gly Ile Met
 260 265 270

Asn Gly Val Asp Gly Leu Val Leu Ala Ser Gly Asn Asp Trp Arg Ala
 275 280 285

Ile Glu Ala Gly Ala His Ala Tyr Ala Ser Leu Thr Gly His Tyr Arg
 290 295 300

Pro Leu Ser Lys Trp Glu Lys Thr Gln Asp Gly Gln Leu Lys Gly Thr
 305 310 315 320

Ile Thr Leu Pro Leu Pro Ile Ala Thr Val Gly Gly Ala Ile Ala Ser
 325 330 335

His Pro Val Ala Gln Val Ser Gln Gln Ile Leu Gly Gln Pro Thr Ala
 340 345 350

Lys Gln Leu Ala Arg Leu Val Ala Ala Val Gly Leu Ala Gln Asn Leu
 355 360 365

Ser Ala Leu Arg Ala Leu Val Thr Thr Gly Ile Gln Gln Gly His Met
 370 375 380

Ala Leu Gln Ala Arg Ser Leu Ala Met Asn Ala Gly Ala Arg Gly Asp
 385 390 395 400

Lys Ile Gln Lys Leu Ala Asp Arg Leu Ile Asn Gln Asp Gln Met Asn
 405 410 415

Leu Ala Thr Ala Arg Ala Leu Leu Lys Asn Met Glu Glu Asp
 420 425 430

<210> 39

<211> 930

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (13)..(930)

<223>

<400> 39

aggattagta aa atg tta ttt gat cgt atc gta gaa gcc ttt ccc gaa agc 51
 Met Leu Phe Asp Arg Ile Val Glu Ala Phe Pro Glu Ser
 1 5 10

aac atc aaa aaa gat gaa ccc ttg tcc tat tac tct tac act cga aca 99
 Asn Ile Lys Lys Asp Glu Pro Leu Ser Tyr Tyr Ser Tyr Thr Arg Thr
 15 20 25

ggt ggc ccg gct gac att ttg att ttc cca gaa tcc atc gat gaa att Gly Gly Pro Ala Asp Ile Leu Ile Phe Pro Glu Ser Ile Asp Glu Ile 30 35 40 45	147
gtg acg att atc aag tgg atc aac caa agt ccg gaa tac caa gct ggc Val Thr Ile Ile Lys Trp Ile Asn Gln Ser Pro Glu Tyr Gln Ala Gly 50 55 60	195
gat ctc ccc ctc act atc tta ggc aat gct agc aac ctg atc gta aaa Asp Leu Pro Leu Thr Ile Leu Gly Asn Ala Ser Asn Leu Ile Val Lys 65 70 75	243
gat ggt ggg ata aga ggg att acc atc att acc acc ggc att aaa acc Asp Gly Gly Ile Arg Gly Ile Thr Ile Ile Thr Thr Gly Ile Lys Thr 80 85 90	291
att tgt cac gaa gag aac cgg atc act gcg ggc gct gga gca gct att Ile Cys His Glu Glu Asn Arg Ile Thr Ala Gly Ala Gly Ala Ala Ile 95 100 105	339
atc gat gtt agc cag gct gcc ttg gac cat agc tta act ggc ttg gaa Ile Asp Val Ser Gln Ala Ala Leu Asp His Ser Leu Thr Gly Leu Glu 110 115 120 125	387
ttc gct tgt ggc ata ccg ggt agt aca ggc ggg gct gtt tac atg aac Phe Ala Cys Gly Ile Pro Gly Ser Thr Gly Gly Ala Val Tyr Met Asn 130 135 140	435
gct ggg gct tac ggt ggg gaa gtc cag cat tgt gtt gaa agt gtc caa Ala Gly Ala Tyr Gly Gly Glu Val Gln His Cys Val Glu Ser Val Gln 145 150 155	483
gtc ttg acc ccg cat ggc cag ttg aag acc tat agt aat gcg gaa atg Val Leu Thr Arg His Gly Gln Leu Lys Thr Tyr Ser Asn Ala Glu Met 160 165 170	531
aac ttc tcc tac cgc cac agt tat ttg atg gaa gaa gac gat ata gta Asn Phe Ser Tyr Arg His Ser Tyr Leu Met Glu Glu Asp Asp Ile Val 175 180 185	579
gtc tcc gtg acc ttt aaa ttg gag tcg ggc gac tac atc act atc aag Val Ser Val Thr Phe Lys Leu Glu Ser Gly Asp Tyr Ile Thr Ile Lys 190 195 200 205	627
gaa aag atg gat gaa tta acc tac ctt aga gaa tcc aaa caa ccg ctg Glu Lys Met Asp Glu Leu Thr Tyr Leu Arg Glu Ser Lys Gln Pro Leu 210 215 220	675
gaa tac ccc tct tgt ggg tca gtc ttt aaa aga cct gaa ggc cac ttt Glu Tyr Pro Ser Cys Gly Ser Val Phe Lys Arg Pro Glu Gly His Phe 225 230 235	723
acg ggg aaa tta atc cag gat gct ggc ctt caa gga ttg gtc cat ggt Thr Gly Lys Leu Ile Gln Asp Ala Gly Leu Gln Gly Leu Val His Gly 240 245 250	771
gga gcc cag gta tcc gaa aaa cat gcc ggt ttt atc att aat ata ggc	819

Gly Ala Gln Val Ser Glu Lys His Ala Gly Phe Ile Ile Asn Ile Gly
 255 260 265

aat gct acc gcc agc gac tac caa gag ttg atc caa cat atc caa gaa 867
 Asn Ala Thr Ala Ser Asp Tyr Gln Glu Leu Ile Gln His Ile Gln Glu
 270 275 280 285

gaa gtc tac cgg att tac aag gtt aag ctg gaa cgt gaa gtt cgc att 915
 Glu Val Tyr Arg Ile Tyr Lys Val Lys Leu Glu Arg Glu Val Arg Ile
 290 295 300

ata ggg gag gat tag 930
 Ile Gly Glu Asp
 305

<210> 40

<211> 305

<212> PRT

<213> Alloiococcus otitidis

<400> 40

Met Leu Phe Asp Arg Ile Val Glu Ala Phe Pro Glu Ser Asn Ile Lys
 1 5 10 15

Lys Asp Glu Pro Leu Ser Tyr Tyr Ser Tyr Thr Arg Thr Gly Gly Pro
 20 25 30

Ala Asp Ile Leu Ile Phe Pro Glu Ser Ile Asp Glu Ile Val Thr Ile
 35 40 45

Ile Lys Trp Ile Asn Gln Ser Pro Glu Tyr Gln Ala Gly Asp Leu Pro
 50 55 60

Leu Thr Ile Leu Gly Asn Ala Ser Asn Leu Ile Val Lys Asp Gly Gly
 65 70 75 80

Ile Arg Gly Ile Thr Ile Ile Thr Thr Gly Ile Lys Thr Ile Cys His
 85 90 95

Glu Glu Asn Arg Ile Thr Ala Gly Ala Gly Ala Ala Ile Ile Asp Val
 100 105 110

Ser Gln Ala Ala Leu Asp His Ser Leu Thr Gly Leu Glu Phe Ala Cys
 115 120 125

Gly Ile Pro Gly Ser Thr Gly Gly Ala Val Tyr Met Asn Ala Gly Ala
 130 135 140

Tyr Gly Gly Glu Val Gln His Cys Val Glu Ser Val Gln Val Leu Thr
 145 150 155 160

Arg His Gly Gln Leu Lys Thr Tyr Ser Asn Ala Glu Met Asn Phe Ser
 165 170 175

Tyr Arg His Ser Tyr Leu Met Glu Glu Asp Asp Ile Val Val Ser Val
 180 185 190

Thr Phe Lys Leu Glu Ser Gly Asp Tyr Ile Thr Ile Lys Glu Lys Met
 195 200 205

Asp Glu Leu Thr Tyr Leu Arg Glu Ser Lys Gln Pro Leu Glu Tyr Pro
 210 215 220

Ser Cys Gly Ser Val Phe Lys Arg Pro Glu Gly His Phe Thr Gly Lys
 225 230 235 240

Leu Ile Gln Asp Ala Gly Leu Gln Gly Leu Val His Gly Gly Ala Gln
 245 250 255

Val Ser Glu Lys His Ala Gly Phe Ile Ile Asn Ile Gly Asn Ala Thr
 260 265 270

Ala Ser Asp Tyr Gln Glu Leu Ile Gln His Ile Gln Glu Glu Val Tyr
 275 280 285

Arg Ile Tyr Lys Val Lys Leu Glu Arg Glu Val Arg Ile Ile Gly Glu
 290 295 300

Asp
 305

<210> 41
 <211> 1104
 <212> DNA
 <213> *Alloiococcus otitidis*

<220>
 <221> CDS
 <222> (16)..(1104)
 <223>

<400> 41
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[illegible]

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ccc caa aaa ata acc agg acc ccc ttg gac ttc cag tcc ttc tta gac Pro Gln Lys Ile Thr Arg Thr Pro Leu Asp Phe Gln Ser Phe Leu Asp 240 245 250			771
caa tcc caa gag tgt gtc gac ggc ttg gtg gag tct tta agc cag gct Gln Ser Gln Glu Cys Val Asp Gly Leu Val Glu Ser Leu Ser Gln Ala 255 260 265			819
gac tcc cag gca agc tta gct tgg atc caa aag aac cga acc ctc ctc Asp Ser Gln Ala Ser Leu Ala Trp Ile Gln Lys Asn Arg Thr Leu Leu 270 275 280			867
aag gca atg ggc caa agc cgg ggg aaa gtc atc gaa acc aaa gcc ttg Lys Ala Met Gly Gln Ser Arg Gly Lys Val Ile Glu Thr Lys Ala Leu 285 290 295 300			915
acc tac ttg tgc gat att gtc gcg aaa tac gga ggc caa gcc aag tct Thr Tyr Leu Cys Asp Ile Val Ala Lys Tyr Gly Gly Gln Ala Lys Ser 305 310 315			963
tcc ggt gcc ggc ggt gga gat tgt ggc att ggc cta atc aca agg gag Ser Gly Ala Gly Gly Gly Asp Cys Gly Ile Gly Leu Ile Thr Arg Glu 320 325 330			1011
agc cca ata gaa gcc atc tac cgg gaa tgg atg gat gca ggt atc ttg Ser Pro Ile Glu Ala Ile Tyr Arg Glu Trp Met Asp Ala Gly Ile Leu 335 340 345			1059
ccc tta aga cta gac att gta gaa aat ggt gct tgc tat gac taa Pro Leu Arg Leu Asp Ile Val Glu Asn Gly Ala Cys Tyr Asp 350 355 360			1104

<210> 42

<211> 362

<212> PRT

<213> *Alloiococcus otitidis*

<400> 42

Met	Val	Tyr	Ser	Leu	Arg	Ile	Pro	Gly	Lys	Leu	Tyr	Leu	Ala	Gly	Glu
1				5					10					15	

Tyr	Ala	Val	Val	Thr	Pro	Gly	Tyr	Ala	Gly	Ile	Leu	Leu	Thr	Val	Ser
		20						25					30		

Arg	Tyr	Leu	Thr	Leu	Asp	Ile	Trp	Glu	Thr	Ser	Pro	Asp	Gln	Ala	Ser
		35					40					45			

Val	Arg	Ser	Gln	Thr	Tyr	Gly	Asn	Gln	Ala	Tyr	Ala	Trp	Glu	Arg	Leu
		50				55				60					

Asp Gly Ile Phe Ser Phe Lys Asp Trp Ser His Pro Phe His Leu Val
65 70 75 80

Glu Thr Val Ile Gln Thr Val Glu Ala Tyr Ile Glu Ser Leu Ser Leu
85 90 95

Pro Leu Lys Ser Tyr Gly Ile Gln Ile Lys Ser Gln Leu Asp Tyr Gln
100 105 110

Gly Lys Lys Ile Gly Leu Gly Ser Ser Gly Ala Val Thr Ile Ala Val
115 120 125

Ile Arg Gly Leu Ser Leu Leu Tyr Asp Leu His Leu Lys Asp Ile Asp
130 135 140

Ile Phe Lys Leu Ala Ala Ile Ala His Ile Gln Leu Lys Ser Lys Gly
145 150 155 160

Ser Phe Gly Asp Leu Ala Ala Cys Thr Tyr Thr Gly Val Ile Arg Tyr
165 170 175

Gln Ser Leu Asp Arg Glu Trp Leu Gln Glu Gln Ile Ser Asn His Ser
180 185 190

Ile Lys Asp Leu Leu Ala Met Asp Trp Pro Ser Leu Gly Leu Asp Arg
195 200 205

Leu Ser Leu Pro His Asp Leu Arg Leu Leu Ile Gly Trp Thr Gly Gln
210 215 220

Pro Ala Ser Thr Glu Lys Leu Val Gln Ala Val Tyr Pro Gln Lys Ile
225 230 235 240

Thr Arg Thr Pro Leu Asp Phe Gln Ser Phe Leu Asp Gln Ser Gln Glu
245 250 255

Cys Val Asp Gly Leu Val Glu Ser Leu Ser Gln Ala Asp Ser Gln Ala
260 265 270

Ser Leu Ala Trp Ile Gln Lys Asn Arg Thr Leu Leu Lys Ala Met Gly
275 280 285

Gln Ser Arg Gly Lys Val Ile Glu Thr Lys Ala Leu Thr Tyr Leu Cys

290

295

300

Asp Ile Val Ala Lys Tyr Gly Gly Gln Ala Lys Ser Ser Gly Ala Gly
 305 310 315 320

Gly Gly Asp Cys Gly Ile Gly Leu Ile Thr Arg Glu Ser Pro Ile Glu
 325 330 335

Ala Ile Tyr Arg Glu Trp Met Asp Ala Gly Ile Leu Pro Leu Arg Leu
 340 345 350

Asp Ile Val Glu Asn Gly Ala Cys Tyr Asp
 355 360

<210> 43

<211> 1023

<212> DNA

<213> *Alloioioccus otitidis*

<220>

<221> CDS

<222> (13)..(1023)

<223>

<400> 43

gagaagccaa cc atg act aag cag gcc ttt gaa aag aaa aag tta ggc cgg 51
 Met Thr Lys Gln Ala Phe Glu Lys Lys Lys Leu Gly Arg
 1 5 10

att tgc cgg gcc cat acc aac att gcc ttg atc aag tac tgg ggt aag 99
 Ile Cys Arg Ala His Thr Asn Ile Ala Leu Ile Lys Tyr Trp Gly Lys
 15 20 25

gct gat agg gac ttg att atc ccc aat aac aac tcc cta tct tta acc 147
 Ala Asp Arg Asp Leu Ile Ile Pro Asn Asn Asn Ser Leu Ser Leu Thr
 30 35 40 45

ttg gac gct ttt tat acc gat acc cag gta gtt ttt gac cca gac ttg 195
 Leu Asp Ala Phe Tyr Thr Asp Thr Gln Val Val Phe Asp Pro Asp Leu
 50 55 60

gac cag gac caa tta tgg cta gac ggg aaa cag gaa aaa ggg tcc gcc 243
 Asp Gln Asp Gln Leu Trp Leu Asp Gly Lys Gln Glu Lys Gly Ser Ala
 65 70 75

tta acc aag gcc cag gtc atc ctg gac ttg gtt cgg gac caa gcc cag 291
 Leu Thr Lys Ala Gln Val Ile Leu Asp Leu Val Arg Asp Gln Ala Gln
 80 85 90

ctt gac tgg ccg gcc aaa att acc agc cac aac caa gtt gcc act gca 339
 Leu Asp Trp Pro Ala Lys Ile Thr Ser His Asn Gln Val Ala Thr Ala
 95 100 105

gct ggc ttg gct tcc tct gct tct ggt ctg gcc gcc ttg gcg ggt gct Ala Gly Leu Ala Ser Ser Ala Ser Gly Leu Ala Ala Leu Ala Gly Ala 110 115 120 125	387
tca gct gat gct tta gac ctt ggc cta tcc cca act gac ctc tcc cga Ser Ala Asp Ala Leu Asp Leu Gly Leu Ser Pro Thr Asp Leu Ser Arg 130 135 140	435
ttg gcc cgc agg gga tct ggg tct gcc tca cga agt att ttt ggt ggt Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg Ser Ile Phe Gly Gly 145 150 155	483
ttt gtc gag tgg gaa aag ggt cat gat gat agc tct tcc ttt gcc aag Phe Val Glu Trp Glu Lys Gly His Asp Asp Ser Ser Ser Phe Ala Lys 160 165 170	531
ccc atc gac ttg gcc cag tgg gat att gcc atg ctc ttt gtc att gta Pro Ile Asp Leu Ala Gln Trp Asp Ile Ala Met Leu Phe Val Ile Val 175 180 185	579
agc gac cga cca aag gca att tcc tcc agc caa ggc atg caa ttg acc Ser Asp Arg Pro Lys Ala Ile Ser Ser Ser Gln Gly Met Gln Leu Thr 190 195 200 205	627
cag gag acg tcg gac ttt tac cag gcc tgg tta gac agc ctg gac caa Gln Glu Thr Ser Asp Phe Tyr Gln Ala Trp Leu Asp Ser Leu Asp Gln 210 215 220	675
gac cta gca gac atc aag tcc gct atc caa gcc caa gac ctc gac cag Asp Leu Ala Asp Ile Lys Ser Ala Ile Gln Ala Gln Asp Leu Asp Gln 225 230 235	723
gtt ggg tcc att gca gaa aga aat gcc ctg aaa atg cat gcc acc aac Val Gly Ser Ile Ala Glu Arg Asn Ala Leu Lys Met His Ala Thr Asn 240 245 250	771
ctg gca gcc aag ccc ccc ttc acc tat tgg act aaa gaa agt tta gcc Leu Ala Ala Lys Pro Pro Phe Thr Tyr Trp Thr Lys Glu Ser Leu Ala 255 260 265	819
ctg atg cag gaa gta tgg gac cgg cgc aag gct ggc cag tcc ctc tac Leu Met Gln Glu Val Trp Asp Arg Arg Lys Ala Gly Gln Ser Leu Tyr 270 275 280 285	867
ttc acc atg gac gcc ggc ccc aat gtc aag gtt att ggc agg gaa gct Phe Thr Met Asp Ala Gly Pro Asn Val Lys Val Ile Gly Arg Glu Ala 290 295 300	915
gac ctt aaa gcc ttc aaa gca gac ctc agc caa gac tgg ccc gac aag Asp Leu Lys Ala Phe Lys Ala Asp Leu Ser Gln Asp Trp Pro Asp Lys 305 310 315	963
cat ctt gtc tta gct aaa ccg ggt cca ggc ctg gcc ttt att gat gga His Leu Val Leu Ala Lys Pro Gly Pro Gly Leu Ala Phe Ile Asp Gly 320 325 330	1011

1023

cct ttg aac tag
Pro Leu Asn
335

<210> 44
<211> 336
<212> PRT
<213> *Alloioioccus otitidis*

<400> 44
Met Thr Lys Gln Ala Phe Glu Lys Lys Lys Leu Gly Arg Ile Cys Arg
1 5 10 15

Ala His Thr Asn Ile Ala Leu Ile Lys Tyr Trp Gly Lys Ala Asp Arg
20 25 30

Asp Leu Ile Ile Pro Asn Asn Asn Ser Leu Ser Leu Thr Leu Asp Ala
35 40 45

Phe Tyr Thr Asp Thr Gln Val Val Phe Asp Pro Asp Leu Asp Gln Asp
50 55 60

Gln Leu Trp Leu Asp Gly Lys Gln Glu Lys Gly Ser Ala Leu Thr Lys
65 70 75 80

Ala Gln Val Ile Leu Asp Leu Val Arg Asp Gln Ala Gln Leu Asp Trp
85 90 95

Pro Ala Lys Ile Thr Ser His Asn Gln Val Ala Thr Ala Ala Gly Leu
100 105 110

Ala Ser Ser Ala Ser Gly Leu Ala Ala Leu Ala Gly Ala Ser Ala Asp
115 120 125

Ala Leu Asp Leu Gly Leu Ser Pro Thr Asp Leu Ser Arg Leu Ala Arg
130 135 140

Arg Gly Ser Gly Ser Ala Ser Arg Ser Ile Phe Gly Gly Phe Val Glu
145 150 155 160

Trp Glu Lys Gly His Asp Asp Ser Ser Ser Phe Ala Lys Pro Ile Asp
165 170 175

Leu Ala Gln Trp Asp Ile Ala Met Leu Phe Val Ile Val Ser Asp Arg
180 185 190

Pro Lys Ala Ile Ser Ser Ser Gln Gly Met Gln Leu Thr Gln Glu Thr
 195 200 205

Ser Asp Phe Tyr Gln Ala Trp Leu Asp Ser Leu Asp Gln Asp Leu Ala
 210 215 220

Asp Ile Lys Ser Ala Ile Gln Ala Gln Asp Leu Asp Gln Val Gly Ser
 225 230 235 240

Ile Ala Glu Arg Asn Ala Leu Lys Met His Ala Thr Asn Leu Ala Ala
 245 250 255

Lys Pro Pro Phe Thr Tyr Trp Thr Lys Glu Ser Leu Ala Leu Met Gln
 260 265 270

Glu Val Trp Asp Arg Arg Lys Ala Gly Gln Ser Leu Tyr Phe Thr Met
 275 280 285

Asp Ala Gly Pro Asn Val Lys Val Ile Gly Arg Glu Ala Asp Leu Lys
 290 295 300

Ala Phe Lys Ala Asp Leu Ser Gln Asp Trp Pro Asp Lys His Leu Val
 305 310 315 320

Leu Ala Lys Pro Gly Pro Gly Leu Ala Phe Ile Asp Gly Pro Leu Asn
 325 330 335

<210> 45

<211> 981

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (28)..(981)

<223>

<400> 45

acaaaaatag acaaaggaga caaaagg atg acg ctt gtt aaa aat gta gcc aaa 54
 Met Thr Leu Val Lys Asn Val Ala Lys
 1 5

ggc act gcc cat ggt aaa att att tta atc ggt gag cat gct gtt gtc 102

Gly Thr Ala His Gly Lys Ile Ile Leu Ile Gly Glu His Ala Val Val	
10 15 20 25	
tat aac atg ccg gcc atc gcc ctc cct ttt acc aca gcc acc atc acc	150
Tyr Asn Met Pro Ala Ile Ala Leu Pro Phe Thr Thr Ala Thr Ile Thr	
30 35 40	
gtt gaa gtt agt cct tac caa ggc aaa agc tat cta gaa agt gct tgc	198
Val Glu Val Ser Pro Tyr Gln Gly Lys Ser Tyr Leu Glu Ser Ala Cys	
45 50 55	
tac tgc gga tct tta gac caa gcg ccc ggg gac ttg gca ggg ctt caa	246
Tyr Cys Gly Ser Leu Asp Gln Ala Pro Gly Asp Leu Ala Gly Leu Gln	
60 65 70	
gcc tgt ttg aca gcg gtt tgt gcc gac tta gac cag tcc agc gac cac	294
Ala Cys Leu Thr Ala Val Cys Ala Asp Leu Asp Gln Ser Ser Asp His	
75 80 85	
ttg tat atc aag gtc gac agc atg atc cct gct gaa aga gga atg ggg	342
Leu Tyr Ile Lys Val Asp Ser Met Ile Pro Ala Glu Arg Gly Met Gly	
90 95 100 105	
tcc agt gct gct gtg gcc acc gcc tta gtc aag gcc ctc ttt cac tac	390
Ser Ser Ala Ala Val Ala Thr Ala Leu Val Lys Ala Leu Phe His Tyr	
110 115 120	
ttc caa gtc gac tta agc agt gaa gcc ctc tca gcc tat gtc gag att	438
Phe Gln Val Asp Leu Ser Ser Glu Ala Leu Ser Ala Tyr Val Glu Ile	
125 130 135	
gcc gaa aaa att acc cat ggc aag cca tcg ggt ctg gat gct aca gtc	486
Ala Glu Lys Ile Thr His Gly Lys Pro Ser Gly Leu Asp Ala Thr Val	
140 145 150	
gtc aac tcc att gcc ccc gtt tat ttt aaa cgc aac cag ctt ccc aag	534
Val Asn Ser Ile Ala Pro Val Tyr Phe Lys Arg Asn Gln Leu Pro Lys	
155 160 165	
gcc atc cct tta aat gtt gac ggc tat tta att gca gcc gat act ggg	582
Ala Ile Pro Leu Asn Val Asp Gly Tyr Leu Ile Ala Ala Asp Thr Gly	
170 175 180 185	
att aag ggc cac acg aaa gaa gcc gtt ggg gat gtg gcg aag ctg gtt	630
Ile Lys Gly His Thr Lys Glu Ala Val Gly Asp Val Ala Lys Leu Val	
190 195 200	
gaa act gcc aag gtt caa acc atg gac att gtc cac cac ctc ggc cag	678
Glu Thr Ala Lys Val Gln Thr Met Asp Ile Val His His Leu Gly Gln	
205 210 215	
ctt acc cac cag gct aaa aaa gca atc atg acc aat aac ctc cct ggc	726
Leu Thr His Gln Ala Lys Lys Ala Ile Met Thr Asn Asn Leu Pro Gly	
220 225 230	
tta ggg gag att ttg aac cag tcc cac caa ctc tta aag gat tta act	774
Leu Gly Glu Ile Leu Asn Gln Ser His Gln Leu Leu Lys Asp Leu Thr	

235 240 245

gtc agc aat ccc aag tta gac caa ctt gtc caa gca gcc caa gat gct 822
Val Ser Asn Pro Lys Leu Asp Gln Leu Val Gln Ala Ala Gln Asp Ala
250 255 260 265

gga gct tgc gga gct aag tta acc ggt ggg ggc cgg ggt ggt tgc atg 870
Gly Ala Cys Gly Ala Lys Leu Thr Gly Gly Gly Arg Gly Gly Cys Met
270 275 280

att gcc cta gcc caa agc aac cag gat gcc tcc aat att gcc caa aaa 918
Ile Ala Leu Ala Gln Ser Asn Gln Asp Ala Ser Asn Ile Ala Gln Lys
285 290 295

ttg gaa aaa gcg gga gcc att gaa acc tgg atc cac ccc tta gga gaa 966
Leu Glu Lys Ala Gly Ala Ile Glu Thr Trp Ile His Pro Leu Gly Glu
300 305 310

gcc aac cat gac taa 981
Ala Asn His Asp
315

<210> 46
<211> 317
<212> PRT
<213> *Alloiococcus otitidis*

<400> 46
Met Thr Leu Val Lys Asn Val Ala Lys Gly Thr Ala His Gly Lys Ile
1 5 10 15

Ile Leu Ile Gly Glu His Ala Val Val Tyr Asn Met Pro Ala Ile Ala
20 25 30

Leu Pro Phe Thr Thr Ala Thr Ile Thr Val Glu Val Ser Pro Tyr Gln
35 40 45

Gly Lys Ser Tyr Leu Glu Ser Ala Cys Tyr Cys Gly Ser Leu Asp Gln
50 55 60

Ala Pro Gly Asp Leu Ala Gly Leu Gln Ala Cys Leu Thr Ala Val Cys
65 70 75 80

Ala Asp Leu Asp Gln Ser Ser Asp His Leu Tyr Ile Lys Val Asp Ser
85 90 95

Met Ile Pro Ala Glu Arg Gly Met Gly Ser Ser Ala Ala Val Ala Thr
100 105 110

Ala Leu Val Lys Ala Leu Phe His Tyr Phe Gln Val Asp Leu Ser Ser
115 120 125

Glu Ala Leu Ser Ala Tyr Val Glu Ile Ala Glu Lys Ile Thr His Gly
130 135 140

Lys Pro Ser Gly Leu Asp Ala Thr Val Val Asn Ser Ile Ala Pro Val
145 150 155 160

Tyr Phe Lys Arg Asn Gln Leu Pro Lys Ala Ile Pro Leu Asn Val Asp
165 170 175

Gly Tyr Leu Ile Ala Ala Asp Thr Gly Ile Lys Gly His Thr Lys Glu
180 185 190

Ala Val Gly Asp Val Ala Lys Leu Val Glu Thr Ala Lys Val Gln Thr
195 200 205

Met Asp Ile Val His His Leu Gly Gln Leu Thr His Gln Ala Lys Lys
210 215 220

Ala Ile Met Thr Asn Asn Leu Pro Gly Leu Gly Glu Ile Leu Asn Gln
225 230 235 240

Ser His Gln Leu Leu Lys Asp Leu Thr Val Ser Asn Pro Lys Leu Asp
245 250 255

Gln Leu Val Gln Ala Ala Gln Asp Ala Gly Ala Cys Gly Ala Lys Leu
260 265 270

Thr Gly Gly Gly Arg Gly Gly Cys Met Ile Ala Leu Ala Gln Ser Asn
275 280 285

Gln Asp Ala Ser Asn Ile Ala Gln Lys Leu Glu Lys Ala Gly Ala Ile
290 295 300

Glu Thr Trp Ile His Pro Leu Gly Glu Ala Asn His Asp
305 310 315

<210> 47

<211> 975

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (46) .. (975)

<223>

<400> 47

agaatcaaatt ttgtttttaa attatcagct tttggagggtc agaac atg aac aat tcc 57
 Met Asn Asn Ser
 1

cgt att ttt tta gtc tat gac cgt aaa gac tgg cag tct ctt aga gaa 105
 Arg Ile Phe Leu Val Tyr Asp Arg Lys Asp Trp Gln Ser Leu Arg Glu
 5 10 15 20

aat gcc agc ctt tct tta acg gaa aaa aac cta aat aac ttg cgt gca 153
 Asn Ala Ser Leu Ser Leu Thr Glu Lys Asn Leu Asn Asn Leu Arg Ala
 25 30 35

gtg aat gac gtc ata tcg atg gaa gat gtc cga gaa gtt tac gtc ccc 201
 Val Asn Asp Val Ile Ser Met Glu Asp Val Arg Glu Val Tyr Val Pro
 40 45 50

att atc caa tta ctg gat gtc tac ata aaa agt tac tac cgc cac cag 249
 Ile Ile Gln Leu Leu Asp Val Tyr Ile Lys Ser Tyr Tyr Arg His Gln
 55 60 65

gct tcc ttg atc aat tac ttg aac ctg gac cag cct aaa aag tac caa 297
 Ala Ser Leu Ile Asn Tyr Leu Asn Leu Asp Gln Pro Lys Lys Tyr Gln
 70 75 80

ccc tat gtg att ggg att gca ggg agc gtg gct gtg ggc aag tct acg 345
 Pro Tyr Val Ile Gly Ile Ala Gly Ser Val Ala Val Gly Lys Ser Thr
 85 90 95 100

gtt gcc agg ctt ctt aag tcc ctc ttg agc gac tac tat ccg gaa aaa 393
 Val Ala Arg Leu Leu Lys Ser Leu Leu Ser Asp Tyr Tyr Pro Glu Lys
 105 110 115

aag gta gac ctc ctc aca aca gat ggc ttc ctt tat ccg aat aag att 441
 Lys Val Asp Leu Leu Thr Thr Asp Gly Phe Leu Tyr Pro Asn Lys Ile
 120 125 130

tta aaa gag cga gat atc atg gac cgc aag ggt ttt ccc gaa agc tat 489
 Leu Lys Glu Arg Asp Ile Met Asp Arg Lys Gly Phe Pro Glu Ser Tyr
 135 140 145

gat atg aaa cgt ttg att aac ttt atg acc gat gtc aaa aat aat gtt 537
 Asp Met Lys Arg Leu Ile Asn Phe Met Thr Asp Val Lys Asn Asn Val
 150 155 160

ccc aac atc cag gtg ccc aag tat tcc cac caa gtt tac gac ata gta 585
 Pro Asn Ile Gln Val Pro Lys Tyr Ser His Gln Val Tyr Asp Ile Val
 165 170 175 180

gaa ggg gaa agg ttg acc att aac cag cca gac atc ttg att gtc gaa 633
 Glu Gly Glu Arg Leu Thr Ile Asn Gln Pro Asp Ile Leu Ile Val Glu
 185 190 195

ggg atc aat gtg ctc caa ctt cct tct aat gag aag att ttt gtt agc 681
 Gly Ile Asn Val Leu Gln Leu Pro Ser Asn Glu Lys Ile Phe Val Ser
 200 205 210
 gat ttt ttc gac ttc tcc ttt tat gtg gat gcc tca gaa aat ctg att 729
 Asp Phe Phe Asp Phe Ser Phe Tyr Val Asp Ala Ser Glu Asn Leu Ile
 215 220 225
 gaa aaa tgg tac atg caa cgc ttt ggc acc ttt atg gat acc gcc ttc 777
 Glu Lys Trp Tyr Met Gln Arg Phe Gly Thr Phe Met Asp Thr Ala Phe
 230 235 240
 caa gac ccc aac aac tat tac tac aag ttt aat gac tgg gac cgc aag 825
 Gln Asp Pro Asn Asn Tyr Tyr Tyr Lys Phe Asn Asp Trp Asp Arg Lys
 245 250 255 260
 gaa gct ttt gcc tat gcc aac caa gtt tgg gaa acg gtt aac cta gaa 873
 Glu Ala Phe Ala Tyr Ala Asn Gln Val Trp Glu Thr Val Asn Leu Glu
 265 270 275
 aac ctc agg gaa tat att cta ccc acc cga ctc cgg gct aac ctc atc 921
 Asn Leu Arg Glu Tyr Ile Leu Pro Thr Arg Leu Arg Ala Asn Leu Ile
 280 285 290
 ctc cat aaa acc cat aac cac tac atc gac aag att tta ctc aaa aaa 969
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 295 300 305
 cac tga 975
 His

<210> 48

<211> 309

<212> PRT

<213> *Alloiococcus otitidis*

<400> 48

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Asn Leu Arg Ala Val Asn Asp Val Ile Ser Met Glu Asp Val Arg Glu
 35 40 45

Val Tyr Val Pro Ile Ile Gln Leu Leu Asp Val Tyr Ile Lys Ser Tyr
 50 55 60

Tyr Arg His Gln Ala Ser Leu Ile Asn Tyr Leu Asn Leu Asp Gln Pro
 65 70 75 80

Lys Lys Tyr Gln Pro Tyr Val Ile Gly Ile Ala Gly Ser Val Ala Val
85 90 95

Gly Lys Ser Thr Val Ala Arg Leu Leu Lys Ser Leu Leu Ser Asp Tyr
100 105 110

Tyr Pro Glu Lys Lys Val Asp Leu Leu Thr Thr Asp Gly Phe Leu Tyr
115 120 125

Pro Asn Lys Ile Leu Lys Glu Arg Asp Ile Met Asp Arg Lys Gly Phe
130 135 140

Pro Glu Ser Tyr Asp Met Lys Arg Leu Ile Asn Phe Met Thr Asp Val
145 150 155 160

Lys Asn Asn Val Pro Asn Ile Gln Val Pro Lys Tyr Ser His Gln Val
165 170 175

Tyr Asp Ile Val Glu Gly Glu Arg Leu Thr Ile Asn Gln Pro Asp Ile
180 185 190

Leu Ile Val Glu Gly Ile Asn Val Leu Gln Leu Pro Ser Asn Glu Lys
195 200 205

Ile Phe Val Ser Asp Phe Phe Asp Phe Ser Phe Tyr Val Asp Ala Ser
210 215 220

Glu Asn Leu Ile Glu Lys Trp Tyr Met Gln Arg Phe Gly Thr Phe Met
225 230 235 240

Asp Thr Ala Phe Gln Asp Pro Asn Asn Tyr Tyr Tyr Lys Phe Asn Asp
245 250 255

Trp Asp Arg Lys Glu Ala Phe Ala Tyr Ala Asn Gln Val Trp Glu Thr
260 265 270

Val Asn Leu Glu Asn Leu Arg Glu Tyr Ile Leu Pro Thr Arg Leu Arg
275 280 285

Ala Asn Leu Ile Leu His Lys Thr His Asn His Tyr Ile Asp Lys Ile
290 295 300

Leu Leu Lys Lys His
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<210> 49
<211> 846
<212> DNA
<213> *Alloioicoccus otitidis*

<220>
<221> CDS
<222> (7)..(846)
<223>

<400> 49
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1 5 10

gtc caa gcc caa att aac ccc aat gag gaa att cgc cgg acc att gac 96
Val Gln Ala Gln Ile Asn Pro Asn Glu Glu Ile Arg Arg Thr Ile Asp
15 20 25 30

ttt atc aag gac tat ctc cag gcc cac ccc ttc ttt gaa tcc tta atc 144
Phe Ile Lys Asp Tyr Leu Gln Ala His Pro Phe Phe Glu Ser Leu Ile
35 40 45

ttg ggc atc tcc ggt ggc cag gat tcc acc ctc ctg ggt aag cta gcc 192
Leu Gly Ile Ser Gly Gly Gln Asp Ser Thr Leu Leu Gly Lys Leu Ala
50 55 60

cag atg gcc tgc ctt gaa ctg agg gaa gag gag ggg tct gac aag cca 240
Gln Met Ala Cys Leu Glu Leu Arg Glu Glu Glu Gly Ser Asp Lys Pro
65 70 75

att ttt att ggt atc cgc cta cct tat ggg gat caa ttt gat gaa gca 288
Ile Phe Ile Gly Ile Arg Leu Pro Tyr Gly Asp Gln Phe Asp Glu Ala
80 85 90

gaa gcc cag caa gcc ctc aat tgg atc cag cct gac cag gct ctg acc 336
Glu Ala Gln Gln Ala Leu Asn Trp Ile Gln Pro Asp Gln Ala Leu Thr
95 100 105 110

att aat atc aaa gag tcc gtt gat ggc ctg gtt gac act ttg gcc ggc 384
Ile Asn Ile Lys Glu Ser Val Asp Gly Leu Val Asp Thr Leu Ala Gly
115 120 125

caa ggc att gaa gtt tct gac ttt aac aag ggc aat atc aaa gct cgg 432
Gln Gly Ile Glu Val Ser Asp Phe Asn Lys Gly Asn Ile Lys Ala Arg
130 135 140

atc cga atg gtg gcc caa tat ggc gta gcg ggt cac ttc cac ggg gcg 480
Ile Arg Met Val Ala Gln Tyr Gly Val Ala Gly His Phe His Gly Ala
145 150 155

gtg tta gga tct gac cat tca gcc gaa aat gta act ggc ttt ttc acc 528

Val	Leu	Gly	Ser	Asp	His	Ser	Ala	Glu	Asn	Val	Thr	Gly	Phe	Phe	Thr	
160						165				170						
aag	cat	ggg	gac	ggc	gct	agt	gac	ctc	aac	cct	ctt	ttc	cgc	cta	aat	576
Lys	His	Gly	Asp	Gly	Ala	Ser	Asp	Leu	Asn	Pro	Leu	Phe	Arg	Leu	Asn	
175				180					185						190	
aaa	cgt	cag	gga	cgg	gcc	ctg	ctt	gag	gaa	tta	ggg	tcc	cct	aag	aac	624
Lys	Arg	Gln	Gly	Arg	Ala	Leu	Leu	Glu	Glu	Leu	Gly	Ser	Pro	Lys	Asn	
			195					200						205		
ttg	tac	caa	aag	acc	ccc	aca	gct	gat	ttg	gaa	gaa	gac	cag	ccc	ggc	672
Leu	Tyr	Gln	Lys	Thr	Pro	Thr	Ala	Asp	Leu	Glu	Glu	Asp	Gln	Pro	Gly	
		210					215						220			
ttg	tca	gat	gaa	gac	aag	tta	ggg	gtt	tct	tat	gaa	gcc	att	gat	gac	720
Leu	Ser	Asp	Glu	Asp	Lys	Leu	Gly	Val	Ser	Tyr	Glu	Ala	Ile	Asp	Asp	
		225					230					235				
tac	ttg	gag	ggc	aag	cca	gtt	agc	cag	gag	gac	cag	gca	acc	atc	gaa	768
Tyr	Leu	Glu	Gly	Lys	Pro	Val	Ser	Gln	Glu	Asp	Gln	Ala	Thr	Ile	Glu	
	240					245					250					
aaa	tgg	tat	caa	caa	acg	gcc	cac	aag	cgc	cac	ttg	ccg	gtg	act	atc	816
Lys	Trp	Tyr	Gln	Gln	Thr	Ala	His	Lys	Arg	His	Leu	Pro	Val	Thr	Ile	
255				260					265						270	
ttt	gat	gat	ttt	tgg	aaa	gaa	aaa	aat	tag							846
Phe	Asp	Asp	Phe	Trp	Lys	Glu	Lys	Asn								
			275													

<210> 50

<211> 279

<212> PRT

<213> *Alloioioccocus otitidis*

<400> 50

Met	Gly	Asp	Asp	Leu	Arg	Glu	Glu	Ile	Leu	Asp	Arg	Met	Lys	Val	Gln	
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Ala	Gln	Ile	Asn	Pro	Asn	Glu	Glu	Ile	Arg	Arg	Thr	Ile	Asp	Phe	Ile	
		20						25					30			

Lys	Asp	Tyr	Leu	Gln	Ala	His	Pro	Phe	Phe	Glu	Ser	Leu	Ile	Leu	Gly	
	35						40					45				

Ile	Ser	Gly	Gly	Gln	Asp	Ser	Thr	Leu	Leu	Gly	Lys	Leu	Ala	Gln	Met	
	50					55					60					

Ala	Cys	Leu	Glu	Leu	Arg	Glu	Glu	Glu	Gly	Ser	Asp	Lys	Pro	Ile	Phe	
65					70					75					80	

Ile Gly Ile Arg Leu Pro Tyr Gly Asp Gln Phe Asp Glu Ala Glu Ala
85 90 95

Gln Gln Ala Leu Asn Trp Ile Gln Pro Asp Gln Ala Leu Thr Ile Asn
100 105 110

Ile Lys Glu Ser Val Asp Gly Leu Val Asp Thr Leu Ala Gly Gln Gly
115 120 125

Ile Glu Val Ser Asp Phe Asn Lys Gly Asn Ile Lys Ala Arg Ile Arg
130 135 140

Met Val Ala Gln Tyr Gly Val Ala Gly His Phe His Gly Ala Val Leu
145 150 155 160

Gly Ser Asp His Ser Ala Glu Asn Val Thr Gly Phe Phe Thr Lys His
165 170 175

Gly Asp Gly Ala Ser Asp Leu Asn Pro Leu Phe Arg Leu Asn Lys Arg
180 185 190

Gln Gly Arg Ala Leu Leu Glu Glu Leu Gly Ser Pro Lys Asn Leu Tyr
195 200 205

Gln Lys Thr Pro Thr Ala Asp Leu Glu Glu Asp Gln Pro Gly Leu Ser
210 215 220

Asp Glu Asp Lys Leu Gly Val Ser Tyr Glu Ala Ile Asp Asp Tyr Leu
225 230 235 240

Glu Gly Lys Pro Val Ser Gln Glu Asp Gln Ala Thr Ile Glu Lys Trp
245 250 255

Tyr Gln Gln Thr Ala His Lys Arg His Leu Pro Val Thr Ile Phe Asp
260 265 270

Asp Phe Trp Lys Glu Lys Asn
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<210> 51

<211> 843

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (7)..(843)

<223>

<400> 51

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gtg ggt ggc ttc acc ctg gtc aaa gaa gcc atg aag caa ttg cca aat	96
Val Gly Gly Phe Thr Leu Val Lys Glu Ala Met Lys Gln Leu Pro Asn	
15 20 25 30	
gaa caa ttt tac tat ctg gga gac acc gcc cgg tca cct tat gga cct	144
Glu Gln Phe Tyr Tyr Leu Gly Asp Thr Ala Arg Ser Pro Tyr Gly Pro	
35 40 45	
aaa gac atg gcc act gtc aag gca tat gcc ttt gaa ctt gcc aat tac	192
Lys Asp Met Ala Thr Val Lys Ala Tyr Ala Phe Glu Leu Ala Asn Tyr	
50 55 60	
ctg gtt aaa aac cac cag atc aaa atc ttg gtg atc gct tgt aat act	240
Leu Val Lys Asn His Gln Ile Lys Ile Leu Val Ile Ala Cys Asn Thr	
65 70 75	
gcg act gtc gct gcc ctc aag gac cta aaa cag gcc ttg ccc atc cca	288
Ala Thr Val Ala Ala Leu Lys Asp Leu Lys Gln Ala Leu Pro Ile Pro	
80 85 90	
gtt tta ggg gtc atc tta cct ggt tgc cga gca gct att aag gct agt	336
Val Leu Gly Val Ile Leu Pro Gly Cys Arg Ala Ala Ile Lys Ala Ser	
95 100 105 110	
gtt aac cat cag att ggg gtt att gcc acc cat ggg acc atc cag tcc	384
Val Asn His Gln Ile Gly Val Ile Ala Thr His Gly Thr Ile Gln Ser	
115 120 125	
ggt cgc tat gag ctt gaa ctt aaa cgg aaa cga ccg gat att gaa gtg	432
Gly Arg Tyr Glu Leu Glu Leu Lys Arg Lys Arg Pro Asp Ile Glu Val	
130 135 140	
aca agt ctg gct tgt ccc gaa ttt gcc ccc atg gta gag gcg gga gac	480
Thr Ser Leu Ala Cys Pro Glu Phe Ala Pro Met Val Glu Ala Gly Asp	
145 150 155	
tac cga tct gtt caa gct agc agt gtg gtg agg aca tcc tta cag gcc	528
Tyr Arg Ser Val Gln Ala Ser Ser Val Val Arg Thr Ser Leu Gln Ala	
160 165 170	
cta gaa gac caa gat ttg gat acc ctt att ttg ggt tgc acc cac tat	576
Leu Glu Asp Gln Asp Leu Asp Thr Leu Ile Leu Gly Cys Thr His Tyr	
175 180 185 190	
ccc att ata aaa gac ctc att caa gac tct att ggc cct ggt atc agc	624
Pro Ile Ile Lys Asp Leu Ile Gln Asp Ser Ile Gly Pro Gly Ile Ser	

195	200	205	
ttg gtt gat cca ggg gcg gaa gct gtg aat gac ttg agt gtc tta tta			672
Leu Val Asp Pro Gly Ala Glu Ala Val Asn Asp Leu Ser Val Leu Leu			
210	215	220	
gac tat tat gac ttg act aat gac cgg ttt aat ccc aac ctg acc cac			720
Asp Tyr Tyr Asp Leu Thr Asn Asp Arg Phe Asn Pro Asn Leu Thr His			
225	230	235	
cat ttt tac acc acg gga gat aaa gcc ggg ttt aag aaa atc gcg gat			768
His Phe Tyr Thr Thr Gly Asp Lys Ala Gly Phe Lys Lys Ile Ala Asp			
240	245	250	
gac tgg ctt gac cac cac aac tac cgg gtt gac cat tta gat tta gag			816
Asp Trp Leu Asp His His Asn Tyr Arg Val Asp His Leu Asp Leu Glu			
255	260	265	270
gag ttg caa gaa gtt aat gga aga taa			843
Glu Leu Gln Glu Val Asn Gly Arg			
275			

<210> 52

<211> 278

<212> PRT

<213> Alloiococcus otitidis

<400> 52

Met	Ile	Met	Tyr	Thr	Asp	Gly	Ile	Gly	Phe	Ile	Asp	Ser	Gly	Val	Gly
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Gly	Phe	Thr	Leu	Val	Lys	Glu	Ala	Met	Lys	Gln	Leu	Pro	Asn	Glu	Gln
			20					25					30		

Phe	Tyr	Tyr	Leu	Gly	Asp	Thr	Ala	Arg	Ser	Pro	Tyr	Gly	Pro	Lys	Asp
		35					40					45			

Met	Ala	Thr	Val	Lys	Ala	Tyr	Ala	Phe	Glu	Leu	Ala	Asn	Tyr	Leu	Val
	50				55						60				

Lys	Asn	His	Gln	Ile	Lys	Ile	Leu	Val	Ile	Ala	Cys	Asn	Thr	Ala	Thr
65					70					75					80

Val	Ala	Ala	Leu	Lys	Asp	Leu	Lys	Gln	Ala	Leu	Pro	Ile	Pro	Val	Leu
			85					90						95	

Gly	Val	Ile	Leu	Pro	Gly	Cys	Arg	Ala	Ala	Ile	Lys	Ala	Ser	Val	Asn
			100					105					110		

His Gln Ile Gly Val Ile Ala Thr His Gly Thr Ile Gln Ser Gly Arg
115 120 125

Tyr Glu Leu Glu Leu Lys Arg Lys Arg Pro Asp Ile Glu Val Thr Ser
130 135 140

Leu Ala Cys Pro Glu Phe Ala Pro Met Val Glu Ala Gly Asp Tyr Arg
145 150 155 160

Ser Val Gln Ala Ser Ser Val Val Arg Thr Ser Leu Gln Ala Leu Glu
165 170 175

Asp Gln Asp Leu Asp Thr Leu Ile Leu Gly Cys Thr His Tyr Pro Ile
180 185 190

Ile Lys Asp Leu Ile Gln Asp Ser Ile Gly Pro Gly Ile Ser Leu Val
195 200 205

Asp Pro Gly Ala Glu Ala Val Asn Asp Leu Ser Val Leu Leu Asp Tyr
210 215 220

Tyr Asp Leu Thr Asn Asp Arg Phe Asn Pro Asn Leu Thr His His Phe
225 230 235 240

Tyr Thr Thr Gly Asp Lys Ala Gly Phe Lys Lys Ile Ala Asp Asp Trp
245 250 255

Leu Asp His His Asn Tyr Arg Val Asp His Leu Asp Leu Glu Glu Leu
260 265 270

Gln Glu Val Asn Gly Arg
275

<210> 53

<211> 957

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (7)..(957)

<223>

<400> 53

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Met Thr Lys Glu Ser Ser Phe Met Val Lys Thr Lys Ile Cys

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tct att tta aat ata aca ccg gat tca ttt tct gat ggt ggg cgc aac Ser Ile Leu Asn Ile Thr Pro Asp Ser Phe Ser Asp Gly Gly Arg Asn 15 20 25 30			96
tat cag gca gac caa gcc ata gct cac gga ctc gac ttg gta gac aag Tyr Gln Ala Asp Gln Ala Ile Ala His Gly Leu Asp Leu Val Asp Lys 35 40 45			144
gga gcg gac atg ttg gat att gga ggt gag tcg acc cgg cct ggt tcc Gly Ala Asp Met Leu Asp Ile Gly Gly Glu Ser Thr Arg Pro Gly Ser 50 55 60			192
agt cca gtc gac ctc caa gat gaa atc gac cgt att gta ccg gtg atc Ser Pro Val Asp Leu Gln Asp Glu Ile Asp Arg Ile Val Pro Val Ile 65 70 75			240
aag gga atc aga gaa aaa agt cag gtt cct att tca gta gat acc tac Lys Gly Ile Arg Glu Lys Ser Gln Val Pro Ile Ser Val Asp Thr Tyr 80 85 90			288
cgg gct cca gtt gcc aaa gcg gct att gat gct ggg gcg gat atc atc Arg Ala Pro Val Ala Lys Ala Ala Ile Asp Ala Gly Ala Asp Ile Ile 95 100 105 110			336
aat gat att acc ggt cta act ggt gat gta gac atg gcc gac ttg cta Asn Asp Ile Thr Gly Leu Thr Gly Asp Val Asp Met Ala Asp Leu Leu 115 120 125			384
gct caa gaa ggg gtt aag gcc att gtc atg ttc aac ccg gtt att gct Ala Gln Glu Gly Val Lys Ala Ile Val Met Phe Asn Pro Val Ile Ala 130 135 140			432
cga cct gac cac cca tct tcc caa aaa ttc aga gat ttc ggg ggc cga Arg Pro Asp His Pro Ser Ser Gln Lys Phe Arg Asp Phe Gly Gly Arg 145 150 155			480
gat ttt ttc acc gat gaa gaa aga gat aaa atg tcc caa gca ccc att Asp Phe Phe Thr Asp Glu Glu Arg Asp Lys Met Ser Gln Ala Pro Ile 160 165 170			528
gaa gag gcc atg atg gtc tac ttt gac aaa gtc ttg aac aag gcc cat Glu Glu Ala Met Met Val Tyr Phe Asp Lys Val Leu Asn Lys Ala His 175 180 185 190			576
caa gct ggg att gac cgg gat aag att tta ctg gac ccg gga att ggc Gln Ala Gly Ile Asp Arg Asp Lys Ile Leu Leu Asp Pro Gly Ile Gly 195 200 205			624
ttt ggc ctg acc aag aag gaa aat tac aag ttg att cac agt gtt gcc Phe Gly Leu Thr Lys Lys Glu Asn Tyr Lys Leu Ile His Ser Val Ala 210 215 220			672
tcg att cat gac aag ggc tac ccg gtc ttt tta gga gtt tcc cgc aaa Ser Ile His Asp Lys Gly Tyr Pro Val Phe Leu Gly Val Ser Arg Lys 225 230 235			720

cgc ttc ttg gtg ggg gaa gtc tcc aag cta ggc atc gaa gcc gac cca 768
 Arg Phe Leu Val Gly Glu Val Ser Lys Leu Gly Ile Glu Ala Asp Pro
 240 245 250
 gag acc caa gca gga ttt tta aac cga gac ctg gct tca gct att att 816
 Glu Thr Gln Ala Gly Phe Leu Asn Arg Asp Leu Ala Ser Ala Ile Ile
 255 260 265 270
 aca gct tac gct agc cat ata ggg gta gac tat gtc cgg gtt cat tcc 864
 Thr Ala Tyr Ala Ser His Ile Gly Val Asp Tyr Val Arg Val His Ser
 275 280 285
 tta gat gaa cac aaa ata gca acc acc att acc cat aat att tta aac 912
 Leu Asp Glu His Lys Ile Ala Thr Thr Ile Thr His Asn Ile Leu Asn
 290 295 300
 agc gat agc tta gat gat cag agc ttt gac caa tat aaa aat taa 957
 Ser Asp Ser Leu Asp Asp Gln Ser Phe Asp Gln Tyr Lys Asn
 305 310 315

<210> 54

<211> 316

<212> PRT

<213> Alloiococcus otitidis

<400> 54

Met Thr Lys Glu Ser Ser Phe Met Val Lys Thr Lys Ile Cys Ser Ile
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Leu Asn Ile Thr Pro Asp Ser Phe Ser Asp Gly Gly Arg Asn Tyr Gln
 20 25 30

Ala Asp Gln Ala Ile Ala His Gly Leu Asp Leu Val Asp Lys Gly Ala
 35 40 45

Asp Met Leu Asp Ile Gly Gly Glu Ser Thr Arg Pro Gly Ser Ser Pro
 50 55 60

Val Asp Leu Gln Asp Glu Ile Asp Arg Ile Val Pro Val Ile Lys Gly
 65 70 75 80

Ile Arg Glu Lys Ser Gln Val Pro Ile Ser Val Asp Thr Tyr Arg Ala
 85 90 95

Pro Val Ala Lys Ala Ala Ile Asp Ala Gly Ala Asp Ile Ile Asn Asp
 100 105 110

Ile Thr Gly Leu Thr Gly Asp Val Asp Met Ala Asp Leu Leu Ala Gln

115 120 125

Glu Gly Val Lys Ala Ile Val Met Phe Asn Pro Val Ile Ala Arg Pro
130 135 140

Asp His Pro Ser Ser Gln Lys Phe Arg Asp Phe Gly Gly Arg Asp Phe
145 150 155 160

Phe Thr Asp Glu Glu Arg Asp Lys Met Ser Gln Ala Pro Ile Glu Glu
165 170 175

Ala Met Met Val Tyr Phe Asp Lys Val Leu Asn Lys Ala His Gln Ala
180 185 190

Gly Ile Asp Arg Asp Lys Ile Leu Leu Asp Pro Gly Ile Gly Phe Gly
195 200 205

Leu Thr Lys Lys Glu Asn Tyr Lys Leu Ile His Ser Val Ala Ser Ile
210 215 220

His Asp Lys Gly Tyr Pro Val Phe Leu Gly Val Ser Arg Lys Arg Phe
225 230 235 240

Leu Val Gly Glu Val Ser Lys Leu Gly Ile Glu Ala Asp Pro Glu Thr
245 250 255

Gln Ala Gly Phe Leu Asn Arg Asp Leu Ala Ser Ala Ile Ile Thr Ala
260 265 270

Tyr Ala Ser His Ile Gly Val Asp Tyr Val Arg Val His Ser Leu Asp
275 280 285

Glu His Lys Ile Ala Thr Thr Ile Thr His Asn Ile Leu Asn Ser Asp
290 295 300

Ser Leu Asp Asp Gln Ser Phe Asp Gln Tyr Lys Asn
305 310 315

<210> 55

<211> 561

<212> DNA

<213> Alloiococcus otitidis

<220>

<221> CDS

<222> (28)..(561)

<223>

<400> 55

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                  1                      5

gga gag ata act atg ata gcc tac gtt tgg gcc caa gat gag caa gga      102
Gly Glu Ile Thr Met Ile Ala Tyr Val Trp Ala Gln Asp Glu Gln Gly
10                      15                      20                      25

atc att ggt aaa gac aag gtt ttg cct tgg gaa ttg tcc aat gac tta      150
Ile Ile Gly Lys Asp Lys Val Leu Pro Trp Glu Leu Ser Asn Asp Leu
                      30                      35                      40

aag cat ttt aaa aaa gtt aca gaa ggt cac acc atc ctg atg ggc cgg      198
Lys His Phe Lys Lys Val Thr Glu Gly His Thr Ile Leu Met Gly Arg
                      45                      50                      55

aag acc ttt gaa gga atg gat aaa aag ccc ctc cct aac cga aaa acc      246
Lys Thr Phe Glu Gly Met Asp Lys Lys Pro Leu Pro Asn Arg Lys Thr
                      60                      65                      70

ttg gta ttg acc cgc caa gat gac tac caa gct ggg gac gac cag gtt      294
Leu Val Leu Thr Arg Gln Asp Asp Tyr Gln Ala Gly Asp Asp Gln Val
                      75                      80                      85

gaa gtc gtc cac tcc aaa gac cag gcc ttg act tat gcg tca ggt cat      342
Glu Val Val His Ser Lys Asp Gln Ala Leu Thr Tyr Ala Ser Gly His
90                      95                      100                      105

ggg gtg gac ctc tat gtg att ggt ggg gcc ggc att ttc gac ttg ttt      390
Gly Val Asp Leu Tyr Val Ile Gly Gly Ala Gly Ile Phe Asp Leu Phe
                      110                      115                      120

ctg gac caa gtt gat gtt ctc cac caa aca gtt atc cac gag agc ttt      438
Leu Asp Gln Val Asp Val Leu His Gln Thr Val Ile His Glu Ser Phe
                      125                      130                      135

gat ggt gac acc acc atg cca gac att gac tgg gac agc ttt aat cag      486
Asp Gly Asp Thr Thr Met Pro Asp Ile Asp Trp Asp Ser Phe Asn Gln
                      140                      145                      150

gtg tct aaa gct tat tat gac cag gct gac ggt cac aac cac tcc cac      534
Val Ser Lys Ala Tyr Tyr Asp Gln Ala Asp Gly His Asn His Ser His
                      155                      160                      165

acc att tat gaa tac aga aga aaa taa      561
Thr Ile Tyr Glu Tyr Arg Arg Lys
170                      175

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<210> 56

<211> 177

<212> PRT

<213> Alloiococcus otitidis

<400> 56

Met Gly Ile Phe Lys Pro Ile Cys Ile Gly Glu Ile Thr Met Ile Ala
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Tyr Val Trp Ala Gln Asp Glu Gln Gly Ile Ile Gly Lys Asp Lys Val
20 25 30

Leu Pro Trp Glu Leu Ser Asn Asp Leu Lys His Phe Lys Lys Val Thr
35 40 45

Glu Gly His Thr Ile Leu Met Gly Arg Lys Thr Phe Glu Gly Met Asp
50 55 60

Lys Lys Pro Leu Pro Asn Arg Lys Thr Leu Val Leu Thr Arg Gln Asp
65 70 75 80

Asp Tyr Gln Ala Gly Asp Asp Gln Val Glu Val Val His Ser Lys Asp
85 90 95

Gln Ala Leu Thr Tyr Ala Ser Gly His Gly Val Asp Leu Tyr Val Ile
100 105 110

Gly Gly Ala Gly Ile Phe Asp Leu Phe Leu Asp Gln Val Asp Val Leu
115 120 125

His Gln Thr Val Ile His Glu Ser Phe Asp Gly Asp Thr Thr Met Pro
130 135 140

Asp Ile Asp Trp Asp Ser Phe Asn Gln Val Ser Lys Ala Tyr Tyr Asp
145 150 155 160

Gln Ala Asp Gly His Asn His Ser His Thr Ile Tyr Glu Tyr Arg Arg
165 170 175

Lys

<210> 57

<211> 1968

<212> DNA

<213> Alloiococcus otitidis

<220>

<221> CDS

<222> (7) .. (1968)

<223>

<400> 57

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Met Thr Lys Glu Ser Tyr Asn Asp Ser Ser Ile Thr Ile Leu	
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aag ggc tta gac gcc gtt aag aaa aga cca ggc atg tat atc ggg tca	96
Lys Gly Leu Asp Ala Val Lys Lys Arg Pro Gly Met Tyr Ile Gly Ser	
15 20 25 30	
acc gat gcc agg ggt ttg cac cac ctg gtt tat gaa att acc gat aat	144
Thr Asp Ala Arg Gly Leu His His Leu Val Tyr Glu Ile Thr Asp Asn	
35 40 45	
gct att gat gag gtt ttg gct ggc tac gct gat gaa att gaa gtc aag	192
Ala Ile Asp Glu Val Leu Ala Gly Tyr Ala Asp Glu Ile Glu Val Lys	
50 55 60	
atc cac acg gac gcc tcg gtt tcg gtc aaa gac aat gga cgg ggc atg	240
Ile His Thr Asp Gly Ser Val Ser Val Lys Asp Asn Gly Arg Gly Met	
65 70 75	
cca acc ggg atg cat gag tca ggc cta ccc acc atc cag gtt atc ttt	288
Pro Thr Gly Met His Glu Ser Gly Leu Pro Thr Ile Gln Val Ile Phe	
80 85 90	
acc gtc ctc cat gcc ggg gga aaa ttt ggc caa gag ggg gcc tac aag	336
Thr Val Leu His Ala Gly Gly Lys Phe Gly Gln Glu Gly Ala Tyr Lys	
95 100 105 110	
tca gcc ggt gga ctc cat ggg gtt ggg gcc tcg gtc gtc aac gcc ttg	384
Ser Ala Gly Gly Leu His Gly Val Gly Ala Ser Val Val Asn Ala Leu	
115 120 125	
tct gat tgg ctc acg gtg ata gtg acc aag gac ggc tat gaa tac cgg	432
Ser Asp Trp Leu Thr Val Ile Val Thr Lys Asp Gly Tyr Glu Tyr Arg	
130 135 140	
caa gac ttt agc caa gga ggc cag gct aaa gga ggc atc cag aag aga	480
Gln Asp Phe Ser Gln Gly Gly Gln Ala Lys Gly Gly Ile Gln Lys Arg	
145 150 155	
aaa att aac cag caa aaa tcc agc acc ctg gtc cac ttc aaa ccc tca	528
Lys Ile Asn Gln Gln Lys Ser Ser Thr Leu Val His Phe Lys Pro Ser	
160 165 170	
ggc caa gtc ttt tcg acc acc gaa ttt aac ttt aac acc atc tgt gag	576
Gly Gln Val Phe Ser Thr Thr Glu Phe Asn Phe Asn Thr Ile Cys Glu	
175 180 185 190	
cgg atg cgg gag tcg gcc ttc ctt gtc aaa ggg acc aag att acc gta	624
Arg Met Arg Glu Ser Ala Phe Leu Val Lys Gly Thr Lys Ile Thr Val	
195 200 205	
gag gac ctg cgc cag gaa gaa agc cag gtc ttc caa ttt aat gaa gga	672

Glu Asp Leu Arg Gln Glu Glu Ser Gln Val Phe Gln Phe Asn Glu Gly	
210 215 220	
att aag gcc ttt gtc gac tac tta aat gag ggc aag gat acc ttg agt	720
Ile Lys Ala Phe Val Asp Tyr Leu Asn Glu Gly Lys Asp Thr Leu Ser	
225 230 235	
cca gta acc tat ttt gaa ggt tct gaa gat gaa att gaa gtt gaa ttt	768
Pro Val Thr Tyr Phe Glu Gly Ser Glu Asp Glu Ile Glu Val Glu Phe	
240 245 250	
gcc ttc caa tac aat gac ggc tat tcg gag acg gtt ctg agt ttt gtc	816
Ala Phe Gln Tyr Asn Asp Gly Tyr Ser Glu Thr Val Leu Ser Phe Val	
255 260 265 270	
aac aat gtc cgt acc cgg gat ggg ggc agc cac gaa act gga gct aag	864
Asn Asn Val Arg Thr Arg Asp Gly Gly Ser His Glu Thr Gly Ala Lys	
275 280 285	
tca gct att acc aag gct ttc aac gac tat gct agg aaa agt ggc tta	912
Ser Ala Ile Thr Lys Ala Phe Asn Asp Tyr Ala Arg Lys Ser Gly Leu	
290 295 300	
ctc aaa gag aaa gac agt aac ttg gaa gga tct gac gtc cgg gaa ggg	960
Leu Lys Glu Lys Asp Ser Asn Leu Glu Gly Ser Asp Val Arg Glu Gly	
305 310 315	
att gcg gtt gtt tta tcc gtc cgt atc cca gaa gag att ctc caa ttt	1008
Ile Ala Val Val Leu Ser Val Arg Ile Pro Glu Glu Ile Leu Gln Phe	
320 325 330	
gaa ggc cag acc aag agc aag tta gga act cct caa gcc cgg acc gcc	1056
Glu Gly Gln Thr Lys Ser Lys Leu Gly Thr Pro Gln Ala Arg Thr Ala	
335 340 345 350	
act gac cag gtt atc tca gaa tcc tta act tac ttc ctg gcc gaa aat	1104
Thr Asp Gln Val Ile Ser Glu Ser Leu Thr Tyr Phe Leu Ala Glu Asn	
355 360 365	
ggg gac ttg tct aag caa ctt att cgc aag gcc atc cga gcc cgg tct	1152
Gly Asp Leu Ser Lys Gln Leu Ile Arg Lys Ala Ile Arg Ala Arg Ser	
370 375 380	
gcc agg gaa gca gct cgc aag gcc aag gac cag tcc cgg aac tct gct	1200
Ala Arg Glu Ala Ala Arg Lys Ala Lys Asp Gln Ser Arg Asn Ser Ala	
385 390 395	
tcc aag aaa aaa gtt gaa act ctc ctg tct ggt aag ttg acc cca gct	1248
Ser Lys Lys Lys Val Glu Thr Leu Leu Ser Gly Lys Leu Thr Pro Ala	
400 405 410	
caa agc aag aac gcc cag aaa aat gaa ctt tac tta gtg gag ggg gat	1296
Gln Ser Lys Asn Ala Gln Lys Asn Glu Leu Tyr Leu Val Glu Gly Asp	
415 420 425 430	
tcg gct ggt ggg tca gcc aag caa ggt agg gac cgg aaa ttc caa gca	1344
Ser Ala Gly Gly Ser Ala Lys Gln Gly Arg Asp Arg Lys Phe Gln Ala	

435	440	445	
att ttg ccc ctg cgt gga aag gtt atc aac aca gaa aaa tct tct ttg Ile Leu Pro Leu Arg Gly Lys Val Ile Asn Thr Glu Lys Ser Ser Leu 450 455 460			1392
gat gat att tta aaa aat gaa gaa att tct acc atg att tat acc atc Asp Asp Ile Leu Lys Asn Glu Glu Ile Ser Thr Met Ile Tyr Thr Ile 465 470 475			1440
ggt gca ggt gct ggg cct gag ttt gat att gaa gct gtt aat tac gat Gly Ala Gly Ala Gly Pro Glu Phe Asp Ile Glu Ala Val Asn Tyr Asp 480 485 490			1488
aag ata gtc att atg act gat gcc gac aca gac ggc gcc cac atc cag Lys Ile Val Ile Met Thr Asp Ala Asp Thr Asp Gly Ala His Ile Gln 495 500 505 510			1536
gtc ctt ctc ctc acc ttc ttt tac cgg tac atg aaa ccc ctg att gaa Val Leu Leu Leu Thr Phe Phe Tyr Arg Tyr Met Lys Pro Leu Ile Glu 515 520 525			1584
gca ggg aag gtc tat att gcc cta ccg ccc ttg tat aag ttg acc aaa Ala Gly Lys Val Tyr Ile Ala Leu Pro Pro Leu Tyr Lys Leu Thr Lys 530 535 540			1632
aag caa gga aag caa gaa aaa aca gcc tat gct tgg act gat gag gag Lys Gln Gly Lys Gln Glu Lys Thr Ala Tyr Ala Trp Thr Asp Glu Glu 545 550 555			1680
ttg gaa gac ctg gtt aaa gat ttt ggc aaa cac tac act ctc cag cgc Leu Glu Asp Leu Val Lys Asp Phe Gly Lys His Tyr Thr Leu Gln Arg 560 565 570			1728
tac aag ggt tta ggc gag atg aat gct gac cag ttg tgg gag acc acc Tyr Lys Gly Leu Gly Glu Met Asn Ala Asp Gln Leu Trp Glu Thr Thr 575 580 585 590			1776
atg gac cca gag acc aga acc ttg atc cgg gtc acc att gaa gac agt Met Asp Pro Glu Thr Arg Thr Leu Ile Arg Val Thr Ile Glu Asp Ser 595 600 605			1824
gaa aag gct gaa aga cgg gtt tcc acc ttg atg ggg acc aag gtg gat Glu Lys Ala Glu Arg Arg Val Ser Thr Leu Met Gly Thr Lys Val Asp 610 615 620			1872
cct aga cgg aag tgg att gaa gac cat att gaa ttc agt ctg gca gaa Pro Arg Arg Lys Trp Ile Glu Asp His Ile Glu Phe Ser Leu Ala Glu 625 630 635			1920
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<210> 58

<211> 653

<212> PRT

<213> Alloiococcus otitidis

<400> 58

Met Thr Lys Glu Ser Tyr Asn Asp Ser Ser Ile Thr Ile Leu Lys Gly
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Leu Asp Ala Val Lys Lys Arg Pro Gly Met Tyr Ile Gly Ser Thr Asp
20 25 30

Ala Arg Gly Leu His His Leu Val Tyr Glu Ile Thr Asp Asn Ala Ile
35 40 45

Asp Glu Val Leu Ala Gly Tyr Ala Asp Glu Ile Glu Val Lys Ile His
50 55 60

Thr Asp Gly Ser Val Ser Val Lys Asp Asn Gly Arg Gly Met Pro Thr
65 70 75 80

Gly Met His Glu Ser Gly Leu Pro Thr Ile Gln Val Ile Phe Thr Val
85 90 95

Leu His Ala Gly Gly Lys Phe Gly Gln Glu Gly Ala Tyr Lys Ser Ala
100 105 110

Gly Gly Leu His Gly Val Gly Ala Ser Val Val Asn Ala Leu Ser Asp
115 120 125

Trp Leu Thr Val Ile Val Thr Lys Asp Gly Tyr Glu Tyr Arg Gln Asp
130 135 140

Phe Ser Gln Gly Gly Gln Ala Lys Gly Gly Ile Gln Lys Arg Lys Ile
145 150 155 160

Asn Gln Gln Lys Ser Ser Thr Leu Val His Phe Lys Pro Ser Gly Gln
165 170 175

Val Phe Ser Thr Thr Glu Phe Asn Phe Asn Thr Ile Cys Glu Arg Met
180 185 190

Arg Glu Ser Ala Phe Leu Val Lys Gly Thr Lys Ile Thr Val Glu Asp
195 200 205

Leu Arg Gln Glu Glu Ser Gln Val Phe Gln Phe Asn Glu Gly Ile Lys

210

215

220

Ala Phe Val Asp Tyr Leu Asn Glu Gly Lys Asp Thr Leu Ser Pro Val
225 230 235 240

Thr Tyr Phe Glu Gly Ser Glu Asp Glu Ile Glu Val Glu Phe Ala Phe
245 250 255

Gln Tyr Asn Asp Gly Tyr Ser Glu Thr Val Leu Ser Phe Val Asn Asn
260 265 270

Val Arg Thr Arg Asp Gly Gly Ser His Glu Thr Gly Ala Lys Ser Ala
275 280 285

Ile Thr Lys Ala Phe Asn Asp Tyr Ala Arg Lys Ser Gly Leu Leu Lys
290 295 300

Glu Lys Asp Ser Asn Leu Glu Gly Ser Asp Val Arg Glu Gly Ile Ala
305 310 315 320

Val Val Leu Ser Val Arg Ile Pro Glu Glu Ile Leu Gln Phe Glu Gly
325 330 335

Gln Thr Lys Ser Lys Leu Gly Thr Pro Gln Ala Arg Thr Ala Thr Asp
340 345 350

Gln Val Ile Ser Glu Ser Leu Thr Tyr Phe Leu Ala Glu Asn Gly Asp
355 360 365

Leu Ser Lys Gln Leu Ile Arg Lys Ala Ile Arg Ala Arg Ser Ala Arg
370 375 380

Glu Ala Ala Arg Lys Ala Lys Asp Gln Ser Arg Asn Ser Ala Ser Lys
385 390 395 400

Lys Lys Val Glu Thr Leu Leu Ser Gly Lys Leu Thr Pro Ala Gln Ser
405 410 415

Lys Asn Ala Gln Lys Asn Glu Leu Tyr Leu Val Glu Gly Asp Ser Ala
420 425 430

Gly Gly Ser Ala Lys Gln Gly Arg Asp Arg Lys Phe Gln Ala Ile Leu
435 440 445

Pro Leu Arg Gly Lys Val Ile Asn Thr Glu Lys Ser Ser Leu Asp Asp
450 455 460

Ile Leu Lys Asn Glu Glu Ile Ser Thr Met Ile Tyr Thr Ile Gly Ala
465 470 475 480

Gly Ala Gly Pro Glu Phe Asp Ile Glu Ala Val Asn Tyr Asp Lys Ile
485 490 495

Val Ile Met Thr Asp Ala Asp Thr Asp Gly Ala His Ile Gln Val Leu
500 505 510

Leu Leu Thr Phe Phe Tyr Arg Tyr Met Lys Pro Leu Ile Glu Ala Gly
515 520 525

Lys Val Tyr Ile Ala Leu Pro Pro Leu Tyr Lys Leu Thr Lys Lys Gln
530 535 540

Gly Lys Gln Glu Lys Thr Ala Tyr Ala Trp Thr Asp Glu Glu Leu Glu
545 550 555 560

Asp Leu Val Lys Asp Phe Gly Lys His Tyr Thr Leu Gln Arg Tyr Lys
565 570 575

Gly Leu Gly Glu Met Asn Ala Asp Gln Leu Trp Glu Thr Thr Met Asp
580 585 590

Pro Glu Thr Arg Thr Leu Ile Arg Val Thr Ile Glu Asp Ser Glu Lys
595 600 605

Ala Glu Arg Arg Val Ser Thr Leu Met Gly Thr Lys Val Asp Pro Arg
610 615 620

Arg Lys Trp Ile Glu Asp His Ile Glu Phe Ser Leu Ala Glu Asp Gly
625 630 635 640

Ser Ile Leu Glu Asn Lys Val Leu Glu Gly Glu Ala Lys
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<211> 2463
<212> DNA

<213> Alloiococcus otitidis

<220>

<221> CDS

<222> (4) .. (2463)

<223>

<400> 59

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gaa gat gtc atg ggg gac cgg ttc ggc cgg tat tcc aag tac att ata	96
Glu Asp Val Met Gly Asp Arg Phe Gly Arg Tyr Ser Lys Tyr Ile Ile	
20 25 30	
cag gaa agg gcc cta ccg gac ttg cgg gac ggt tta aaa ccg gtc caa	144
Gln Glu Arg Ala Leu Pro Asp Leu Arg Asp Gly Leu Lys Pro Val Gln	
35 40 45	
aga cgg atc ctc tat gcc atg cac cag gac aaa aac acc tat gac aag	192
Arg Arg Ile Leu Tyr Ala Met His Gln Asp Lys Asn Thr Tyr Asp Lys	
50 55 60	
gct tac cgg aag tcg gcc aag acg gtg gga aat gtc ata ggg aac tac	240
Ala Tyr Arg Lys Ser Ala Lys Thr Val Gly Asn Val Ile Gly Asn Tyr	
65 70 75	
cac ccc cat ggc gac aca tcc gtt tac gat gcc atg gtt agg ctc agt	288
His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Met Val Arg Leu Ser	
80 85 90 95	
cag cct tgg aag atg cgc cat cct ttg gtt gat atg cac ggg aac aag	336
Gln Pro Trp Lys Met Arg His Pro Leu Val Asp Met His Gly Asn Lys	
100 105 110	
ggg agc atg gac ggg gac cca cca gct gcc atg cgg tac acc gaa gcc	384
Gly Ser Met Asp Gly Asp Pro Pro Ala Ala Met Arg Tyr Thr Glu Ala	
115 120 125	
cgt ctg tct aaa att gct tcc gac ctc ctg gct gat att gat aag gag	432
Arg Leu Ser Lys Ile Ala Ser Asp Leu Leu Ala Asp Ile Asp Lys Glu	
130 135 140	
acg gtg gac cat gtc tta aac ttt gat gac acg acc gag gag ccc acc	480
Thr Val Asp His Val Leu Asn Phe Asp Asp Thr Thr Glu Glu Pro Thr	
145 150 155	
gtc tta ccc gcc cgt ttt ccc aac ctc ttg gtc aat ggg gct agc ggg	528
Val Leu Pro Ala Arg Phe Pro Asn Leu Leu Val Asn Gly Ala Ser Gly	
160 165 170 175	
att tca gcc ggt tat gct act gac ata ccg ccc cat aat ttg agc gag	576
Ile Ser Ala Gly Tyr Ala Thr Asp Ile Pro Pro His Asn Leu Ser Glu	
180 185 190	
gtg att gat gcc acc atc cac tta atc aac cac ccc aat gca agg ctg	624

Val	Ile	Asp	Ala	Thr	Ile	His	Leu	Ile	Asn	His	Pro	Asn	Ala	Arg	Leu		
			195					200					205				
gag	act	ttg	atg	gac	tat	att	caa	gga	cca	gac	ttt	ccg	act	ggg	ggg	672	
Glu	Thr	Leu	Met	Asp	Tyr	Ile	Gln	Gly	Pro	Asp	Phe	Pro	Thr	Gly	Gly		
		210					215					220					
att	atc	caa	ggt	aaa	agt	ggc	ctg	aag	aaa	gcc	tac	caa	acg	ggc	aag	720	
Ile	Ile	Gln	Gly	Lys	Ser	Gly	Leu	Lys	Lys	Ala	Tyr	Gln	Thr	Gly	Lys		
	225					230				235							
gga	aaa	att	atc	atc	cgg	gcc	aaa	gca	gat	att	gag	gcc	atc	cgg	ggt	768	
Gly	Lys	Ile	Ile	Ile	Arg	Ala	Lys	Ala	Asp	Ile	Glu	Ala	Ile	Arg	Gly		
240					245					250					255		
ggc	aaa	tcc	caa	att	gtc	atc	agt	caa	att	cct	tat	gag	gtc	aac	aag	816	
Gly	Lys	Ser	Gln	Ile	Val	Ile	Ser	Gln	Ile	Pro	Tyr	Glu	Val	Asn	Lys		
			260					265						270			
gca	agg	ttg	gtc	caa	aaa	att	gac	gac	atc	cgg	att	aac	aaa	aaa	atc	864	
Ala	Arg	Leu	Val	Gln	Lys	Ile	Asp	Asp	Ile	Arg	Ile	Asn	Lys	Lys	Ile		
		275						280					285				
gac	ggc	att	gcc	gat	gtc	cgg	gat	gaa	agt	gac	cgg	tct	ggc	ttg	cgg	912	
Asp	Gly	Ile	Ala	Asp	Val	Arg	Asp	Glu	Ser	Asp	Arg	Ser	Gly	Leu	Arg		
	290						295					300					
att	gtg	gtc	gaa	acc	aaa	aaa	gat	ggt	gat	ggg	gaa	ggg	atc	tta	acc	960	
Ile	Val	Val	Glu	Thr	Lys	Lys	Asp	Gly	Asp	Gly	Glu	Gly	Ile	Leu	Thr		
	305					310					315						
tac	ctg	ctg	aaa	aac	acc	gac	ctc	cag	gta	act	tat	aac	tta	aat	atg	1008	
Tyr	Leu	Leu	Lys	Asn	Thr	Asp	Leu	Gln	Val	Thr	Tyr	Asn	Leu	Asn	Met		
320				325						330					335		
gta	gcc	att	gat	aaa	aaa	cga	ccc	cag	caa	gtc	tcc	ctc	aag	caa	atc	1056	
Val	Ala	Ile	Asp	Lys	Lys	Arg	Pro	Gln	Gln	Val	Ser	Leu	Lys	Gln	Ile		
			340					345						350			
tta	tct	tct	tac	ttg	gac	cac	aag	cgg	aca	gtg	gtt	caa	aac	cgg	acc	1104	
Leu	Ser	Ser	Tyr	Leu	Asp	His	Lys	Arg	Thr	Val	Val	Gln	Asn	Arg	Thr		
			355					360					365				
cgt	tac	ctc	tta	gcc	aag	gcc	aag	gac	cgc	cag	cac	att	gtc	caa	ggc	1152	
Arg	Tyr	Leu	Leu	Ala	Lys	Ala	Lys	Asp	Arg	Gln	His	Ile	Val	Gln	Gly		
	370						375					380					
ctt	atc	aag	gcc	att	tca	atc	ctg	gat	gac	ttg	atc	caa	acc	atc	cgg	1200	
Leu	Ile	Lys	Ala	Ile	Ser	Ile	Leu	Asp	Asp	Leu	Ile	Gln	Thr	Ile	Arg		
	385					390					395						
gcc	agt	gaa	aac	aag	gcc	aat	gcc	aag	gaa	aat	att	atc	cag	gct	tat	1248	
Ala	Ser	Glu	Asn	Lys	Ala	Asn	Ala	Lys	Glu	Asn	Ile	Ile	Gln	Ala	Tyr		
400					405				410					415			
ggt	ttt	agc	caa	gac	caa	gcc	gaa	gcc	att	gtc	tcc	ctc	cag	ctt	tac	1296	
Gly	Phe	Ser	Gln	Asp	Gln	Ala	Glu	Ala	Ile	Val	Ser	Leu	Gln	Leu	Tyr		

420	425	430	
cgc ttg acc aat aca gat ata aag gac tta caa gca gaa gcc aaa gac Arg Leu Thr Asn Thr Asp Ile Lys Asp Leu Gln Ala Glu Ala Lys Asp 435 440 445			1344
tta gcc caa gcc atc ctg acc tac cag gac ctc tta acc aac aag gcc Leu Ala Gln Ala Ile Leu Thr Tyr Gln Asp Leu Leu Thr Asn Lys Ala 450 455 460			1392
agc ctg gat gct ttg atg aaa gaa gaa ttg aaa gaa gtc aaa caa gca Ser Leu Asp Ala Leu Met Lys Glu Glu Leu Lys Glu Val Lys Gln Ala 465 470 475			1440
tat ggg gag gac cgg cta acc cag gtc caa gac aag atc gaa aaa cta Tyr Gly Glu Asp Arg Leu Thr Gln Val Gln Asp Lys Ile Glu Lys Leu 480 485 490 495			1488
gaa ata gaa acc caa gtc ctg gtc agt gaa gaa gac gtc atg gtt acc Glu Ile Glu Thr Gln Val Leu Val Ser Glu Glu Asp Val Met Val Thr 500 505 510			1536
gtc acc cag gga ggt tac ttg aag cgg acc tcc atc cgg tct tac aag Val Thr Gln Gly Gly Tyr Leu Lys Arg Thr Ser Ile Arg Ser Tyr Lys 515 520 525			1584
gct tcc caa gtg gag gaa ttg ggc cgg cga gaa gac gac ttg gtc atc Ala Ser Gln Val Glu Glu Leu Gly Arg Arg Glu Asp Asp Leu Val Ile 530 535 540			1632
ttt atg caa gag ttg tca acc cta gac caa ctc ctt att ttc acc tcg Phe Met Gln Glu Leu Ser Thr Leu Asp Gln Leu Leu Ile Phe Thr Ser 545 550 555			1680
aaa ggc aat gtg gtc aac cga cca gtc cat gaa tta ccg gac atc aag Lys Gly Asn Val Val Asn Arg Pro Val His Glu Leu Pro Asp Ile Lys 560 565 570 575			1728
tgg aag gat att gga gag cac ttg tca agg acc atc ccc ctt gga gag Trp Lys Asp Ile Gly Glu His Leu Ser Arg Thr Ile Pro Leu Gly Glu 580 585 590			1776
gac gag gaa ttg att aag gtg tac cct tat cgg gaa tta gat gcc ggc Asp Glu Glu Leu Ile Lys Val Tyr Pro Tyr Arg Glu Leu Asp Ala Gly 595 600 605			1824
aag cgc tat gtc ttt atc act cga gat ggc tat atc aaa caa agt cca Lys Arg Tyr Val Phe Ile Thr Arg Asp Gly Tyr Ile Lys Gln Ser Pro 610 615 620			1872
gag acg gaa ttt gag ccc aaa cga act tac aag tct cgg gct tca act Glu Thr Glu Phe Glu Pro Lys Arg Thr Tyr Lys Ser Arg Ala Ser Thr 625 630 635			1920
gcc att aaa tta aaa tca gac caa gat aga ctc cag gca gtc tac tat Ala Ile Lys Leu Lys Ser Asp Gln Asp Arg Leu Gln Ala Val Tyr Tyr 640 645 650 655			1968

att cct gac caa gaa gat tac gat gta ttc cta gcc agc tac aag ggc 2016
 Ile Pro Asp Gln Glu Asp Tyr Asp Val Phe Leu Ala Ser Tyr Lys Gly
 660 665 670

tac ggg ctc aag tat gga cta gaa gaa gtg tca gaa gta ggg gcc cag 2064
 Tyr Gly Leu Lys Tyr Gly Leu Glu Glu Val Ser Glu Val Gly Ala Gln
 675 680 685

gct gca ggc gtc aag tcc atg aac ctg aaa gag ggg gac cat gtc caa 2112
 Ala Ala Gly Val Lys Ser Met Asn Leu Lys Glu Gly Asp His Val Gln
 690 695 700

gat ggt ttg gtc ttt aag cgt aag cag ttc caa gaa gcc ttg ttc att 2160
 Asp Gly Leu Val Phe Lys Arg Lys Gln Phe Gln Glu Ala Leu Phe Ile
 705 710 715

acc cag cga gcc agt gtt aag aaa atg gcc ctc cat gac ttt gac cgg 2208
 Thr Gln Arg Ala Ser Val Lys Lys Met Ala Leu His Asp Phe Asp Arg
 720 725 730 735

act tca cgg gcc aag cgg ggt tta caa atc ctc aga gaa ctg aag cga 2256
 Thr Ser Arg Ala Lys Arg Gly Leu Gln Ile Leu Arg Glu Leu Lys Arg
 740 745 750

aac ccc cac cga atc cag ttt atg atc gga att tca caa aat aaa ttc 2304
 Asn Pro His Arg Ile Gln Phe Met Ile Gly Ile Ser Gln Asn Lys Phe
 755 760 765

ctg gtc aat ctc cta act gat aca aaa aaa cta gta cag ata aac cca 2352
 Leu Val Asn Leu Leu Thr Asp Thr Lys Lys Leu Val Gln Ile Asn Pro
 770 775 780

gat gac tat aca gtt tca aac cgc cat aac aat ggg tct ttt gtc ctg 2400
 Asp Asp Tyr Thr Val Ser Asn Arg His Asn Asn Gly Ser Phe Val Leu
 785 790 795

gac aca agc cga gat ggc aag cct gtt tct tac tat tta agt gat aac 2448
 Asp Thr Ser Arg Asp Gly Lys Pro Val Ser Tyr Tyr Leu Ser Asp Asn
 800 805 810 815

gat tct cac ttg taa 2463
 Asp Ser His Leu

<210> 60

<211> 819

<212> PRT

<213> *Alloiococcus otitidis*

<400> 60

Met Ala Gly Asp Gln Glu Thr Ser Lys Ile Gln Glu Leu Thr Leu Glu
 1 5 10 15

Asp Val Met Gly Asp Arg Phe Gly Arg Tyr Ser Lys Tyr Ile Ile Gln
 20 25 30

Glu Arg Ala Leu Pro Asp Leu Arg Asp Gly Leu Lys Pro Val Gln Arg
35 40 45

Arg Ile Leu Tyr Ala Met His Gln Asp Lys Asn Thr Tyr Asp Lys Ala
50 55 60

Tyr Arg Lys Ser Ala Lys Thr Val Gly Asn Val Ile Gly Asn Tyr His
65 70 75 80

Pro His Gly Asp Thr Ser Val Tyr Asp Ala Met Val Arg Leu Ser Gln
85 90 95

Pro Trp Lys Met Arg His Pro Leu Val Asp Met His Gly Asn Lys Gly
100 105 110

Ser Met Asp Gly Asp Pro Pro Ala Ala Met Arg Tyr Thr Glu Ala Arg
115 120 125

Leu Ser Lys Ile Ala Ser Asp Leu Leu Ala Asp Ile Asp Lys Glu Thr
130 135 140

Val Asp His Val Leu Asn Phe Asp Asp Thr Thr Glu Glu Pro Thr Val
145 150 155 160

Leu Pro Ala Arg Phe Pro Asn Leu Leu Val Asn Gly Ala Ser Gly Ile
165 170 175

Ser Ala Gly Tyr Ala Thr Asp Ile Pro Pro His Asn Leu Ser Glu Val
180 185 190

Ile Asp Ala Thr Ile His Leu Ile Asn His Pro Asn Ala Arg Leu Glu
195 200 205

Thr Leu Met Asp Tyr Ile Gln Gly Pro Asp Phe Pro Thr Gly Gly Ile
210 215 220

Ile Gln Gly Lys Ser Gly Leu Lys Lys Ala Tyr Gln Thr Gly Lys Gly
225 230 235 240

Lys Ile Ile Ile Arg Ala Lys Ala Asp Ile Glu Ala Ile Arg Gly Gly
245 250 255

Lys Ser Gln Ile Val Ile Ser Gln Ile Pro Tyr Glu Val Asn Lys Ala
260 265 270

Arg Leu Val Gln Lys Ile Asp Asp Ile Arg Ile Asn Lys Lys Ile Asp
275 280 285

Gly Ile Ala Asp Val Arg Asp Glu Ser Asp Arg Ser Gly Leu Arg Ile
290 295 300

Val Val Glu Thr Lys Lys Asp Gly Asp Gly Glu Gly Ile Leu Thr Tyr
305 310 315 320

Leu Leu Lys Asn Thr Asp Leu Gln Val Thr Tyr Asn Leu Asn Met Val
325 330 335

Ala Ile Asp Lys Lys Arg Pro Gln Gln Val Ser Leu Lys Gln Ile Leu
340 345 350

Ser Ser Tyr Leu Asp His Lys Arg Thr Val Val Gln Asn Arg Thr Arg
355 360 365

Tyr Leu Leu Ala Lys Ala Lys Asp Arg Gln His Ile Val Gln Gly Leu
370 375 380

Ile Lys Ala Ile Ser Ile Leu Asp Asp Leu Ile Gln Thr Ile Arg Ala
385 390 395 400

Ser Glu Asn Lys Ala Asn Ala Lys Glu Asn Ile Ile Gln Ala Tyr Gly
405 410 415

Phe Ser Gln Asp Gln Ala Glu Ala Ile Val Ser Leu Gln Leu Tyr Arg
420 425 430

Leu Thr Asn Thr Asp Ile Lys Asp Leu Gln Ala Glu Ala Lys Asp Leu
435 440 445

Ala Gln Ala Ile Leu Thr Tyr Gln Asp Leu Leu Thr Asn Lys Ala Ser
450 455 460

Leu Asp Ala Leu Met Lys Glu Glu Leu Lys Glu Val Lys Gln Ala Tyr
465 470 475 480

Gly Glu Asp Arg Leu Thr Gln Val Gln Asp Lys Ile Glu Lys Leu Glu
485 490 495

Ile Glu Thr Gln Val Leu Val Ser Glu Glu Asp Val Met Val Thr Val
500 505 510

Thr Gln Gly Gly Tyr Leu Lys Arg Thr Ser Ile Arg Ser Tyr Lys Ala
515 520 525

Ser Gln Val Glu Glu Leu Gly Arg Arg Glu Asp Asp Leu Val Ile Phe
530 535 540

Met Gln Glu Leu Ser Thr Leu Asp Gln Leu Leu Ile Phe Thr Ser Lys
545 550 555 560

Gly Asn Val Val Asn Arg Pro Val His Glu Leu Pro Asp Ile Lys Trp
565 570 575

Lys Asp Ile Gly Glu His Leu Ser Arg Thr Ile Pro Leu Gly Glu Asp
580 585 590

Glu Glu Leu Ile Lys Val Tyr Pro Tyr Arg Glu Leu Asp Ala Gly Lys
595 600 605

Arg Tyr Val Phe Ile Thr Arg Asp Gly Tyr Ile Lys Gln Ser Pro Glu
610 615 620

Thr Glu Phe Glu Pro Lys Arg Thr Tyr Lys Ser Arg Ala Ser Thr Ala
625 630 635 640

Ile Lys Leu Lys Ser Asp Gln Asp Arg Leu Gln Ala Val Tyr Tyr Ile
645 650 655

Pro Asp Gln Glu Asp Tyr Asp Val Phe Leu Ala Ser Tyr Lys Gly Tyr
660 665 670

Gly Leu Lys Tyr Gly Leu Glu Glu Val Ser Glu Val Gly Ala Gln Ala
675 680 685

Ala Gly Val Lys Ser Met Asn Leu Lys Glu Gly Asp His Val Gln Asp
690 695 700

Gly Leu Val Phe Lys Arg Lys Gln Phe Gln Glu Ala Leu Phe Ile Thr

705 710 715 720

Gln Arg Ala Ser Val Lys Lys Met Ala Leu His Asp Phe Asp Arg Thr
 725 730 735

Ser Arg Ala Lys Arg Gly Leu Gln Ile Leu Arg Glu Leu Lys Arg Asn
 740 745 750

Pro His Arg Ile Gln Phe Met Ile Gly Ile Ser Gln Asn Lys Phe Leu
 755 760 765

Val Asn Leu Leu Thr Asp Thr Lys Lys Leu Val Gln Ile Asn Pro Asp
 770 775 780

Asp Tyr Thr Val Ser Asn Arg His Asn Asn Gly Ser Phe Val Leu Asp
 785 790 795 800

Thr Ser Arg Asp Gly Lys Pro Val Ser Tyr Tyr Leu Ser Asp Asn Asp
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Ser His Leu

<210> 61
 <211> 1113
 <212> DNA
 <213> Alloiococcus otitidis

<220>
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 <222> (4)..(1113)
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<400> 61

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	1				5				10					15		
att	aaa	gac	ttg	aaa	aat	aaa	aaa	gag	tcg	acc	att	tct	tat	att	gac	96
Ile	Lys	Asp	Leu	Lys	Asn	Lys	Lys	Glu	Ser	Thr	Ile	Ser	Tyr	Ile	Asp	
			20					25						30		
ctc	agc	aac	aaa	att	gct	gaa	ccc	ttc	gaa	ctt	gaa	agt	gaa	gcc	atg	144
Leu	Ser	Asn	Lys	Ile	Ala	Glu	Pro	Phe	Glu	Leu	Glu	Ser	Glu	Ala	Met	
			35					40					45			
gac	aag	tta	atc	cag	caa	tta	gaa	gat	gat	ggg	att	ggt	gta	gtt	gac	192
Asp	Lys	Leu	Ile	Gln	Gln	Leu	Glu	Asp	Asp	Gly	Ile	Gly	Val	Val	Asp	
		50					55					60				

caa gac ggt aat ccc ttg gcc aag caa cta gcc aag cag gaa gaa gaa Gln Asp Gly Asn Pro Leu Ala Lys Gln Leu Ala Lys Gln Glu Glu Glu 65 70 75	240
gca gaa aaa gcc aag gat gaa gaa atg ata gcc cca cct ggg gtt aaa Ala Glu Lys Ala Lys Asp Glu Glu Met Ile Ala Pro Pro Gly Val Lys 80 85 90 95	288
att aac gac cct gtc cgg atg tac cta aaa gaa att ggc cgg gta gat Ile Asn Asp Pro Val Arg Met Tyr Leu Lys Glu Ile Gly Arg Val Asp 100 105 110	336
ctt tta gat gct gaa gaa gaa gtg gcc cta gcc aag cgg att gaa gaa Leu Leu Asp Ala Glu Glu Glu Val Ala Leu Ala Lys Arg Ile Glu Glu 115 120 125	384
ggc gat gaa atc gct aaa caa gaa cta gct gag gct aac ttg aga ctg Gly Asp Glu Ile Ala Lys Gln Glu Leu Ala Glu Ala Asn Leu Arg Leu 130 135 140	432
gtt gtc tct att gct aaa cgg tac gtt ggc cgg ggc atg agc ttt ttg Val Val Ser Ile Ala Lys Arg Tyr Val Gly Arg Gly Met Ser Phe Leu 145 150 155	480
gac ttg atc cag gaa ggg aat atg ggg cta atg aag gca gtt gaa aaa Asp Leu Ile Gln Glu Gly Asn Met Gly Leu Met Lys Ala Val Glu Lys 160 165 170 175	528
ttt gac tac gaa aaa ggt ttc aaa ttt tca acc tat gcc acc tgg tgg Phe Asp Tyr Glu Lys Gly Phe Lys Phe Ser Thr Tyr Ala Thr Trp Trp 180 185 190	576
atc cgt caa gcc atc act cgg gcc att gcc gac caa gcc cga acc atc Ile Arg Gln Ala Ile Thr Arg Ala Ile Ala Asp Gln Ala Arg Thr Ile 195 200 205	624
cgg att ccg gtc cac atg gtc gaa act att aac aag ctg gtc cga atc Arg Ile Pro Val His Met Val Glu Thr Ile Asn Lys Leu Val Arg Ile 210 215 220	672
cag cgg cag ctc cta caa gaa cta ggc cgg gaa cca acc cca gaa gaa Gln Arg Gln Leu Leu Gln Glu Leu Gly Arg Glu Pro Thr Pro Glu Glu 225 230 235	720
att ggg gca gag atg gat ttg cca acc gaa aaa gtc aga gat att ttg Ile Gly Ala Glu Met Asp Leu Pro Thr Glu Lys Val Arg Asp Ile Leu 240 245 250 255	768
aaa att tcc caa gaa ccc gtc tcc ctt gaa acc cca att ggg gaa gaa Lys Ile Ser Gln Glu Pro Val Ser Leu Glu Thr Pro Ile Gly Glu Glu 260 265 270	816
gaa gat tcc cac ctg gga gac ttt att gaa gat gat ggg gcc ttg tcg Glu Asp Ser His Leu Gly Asp Phe Ile Glu Asp Asp Gly Ala Leu Ser 275 280 285	864
cca tct gat aat gca gct tat gag ctg ttg aaa ggg gaa ctc aaa gga	912

Pro Ser Asp Asn Ala Ala Tyr Glu Leu Leu Lys Gly Glu Leu Lys Gly
 290 295 300

gtc tta gac acc cta act gac cgg gaa gaa aat gtc ttg cgc ctc cgt 960
 Val Leu Asp Thr Leu Thr Asp Arg Glu Glu Asn Val Leu Arg Leu Arg
 305 310 315

ttt ggc cta gat gat ggc cgt caa cgt act tta gaa gat gtc ggt aag 1008
 Phe Gly Leu Asp Asp Gly Arg Gln Arg Thr Leu Glu Asp Val Gly Lys
 320 325 330 335

gtc ttt ggg gtc acc cgg gag cgg atc cgt caa att gaa gcg aag gcc 1056
 Val Phe Gly Val Thr Arg Glu Arg Ile Arg Gln Ile Glu Ala Lys Ala
 340 345 350

ctc cgc aaa ctc cgc cac cct agc cgg tcc aaa caa tta aaa gac ttt 1104
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tta gaa tag 1113
 Leu Glu

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 <211> 369
 <212> PRT
 <213> *Alloiococcus otitidis*

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Lys Asp Leu Lys Asn Lys Lys Glu Ser Thr Ile Ser Tyr Ile Asp Leu
 20 25 30

Ser Asn Lys Ile Ala Glu Pro Phe Glu Leu Glu Ser Glu Ala Met Asp
 35 40 45

Lys Leu Ile Gln Gln Leu Glu Asp Asp Gly Ile Gly Val Val Asp Gln
 50 55 60

Asp Gly Asn Pro Leu Ala Lys Gln Leu Ala Lys Gln Glu Glu Glu Ala
 65 70 75 80

Glu Lys Ala Lys Asp Glu Glu Met Ile Ala Pro Pro Gly Val Lys Ile
 85 90 95

Asn Asp Pro Val Arg Met Tyr Leu Lys Glu Ile Gly Arg Val Asp Leu
 100 105 110

Leu Asp Ala Glu Glu Glu Val Ala Leu Ala Lys Arg Ile Glu Glu Gly
115 120 125

Asp Glu Ile Ala Lys Gln Glu Leu Ala Glu Ala Asn Leu Arg Leu Val
130 135 140

Val Ser Ile Ala Lys Arg Tyr Val Gly Arg Gly Met Ser Phe Leu Asp
145 150 155 160

Leu Ile Gln Glu Gly Asn Met Gly Leu Met Lys Ala Val Glu Lys Phe
165 170 175

Asp Tyr Glu Lys Gly Phe Lys Phe Ser Thr Tyr Ala Thr Trp Trp Ile
180 185 190

Arg Gln Ala Ile Thr Arg Ala Ile Ala Asp Gln Ala Arg Thr Ile Arg
195 200 205

Ile Pro Val His Met Val Glu Thr Ile Asn Lys Leu Val Arg Ile Gln
210 215 220

Arg Gln Leu Leu Gln Glu Leu Gly Arg Glu Pro Thr Pro Glu Glu Ile
225 230 235 240

Gly Ala Glu Met Asp Leu Pro Thr Glu Lys Val Arg Asp Ile Leu Lys
245 250 255

Ile Ser Gln Glu Pro Val Ser Leu Glu Thr Pro Ile Gly Glu Glu Glu
260 265 270

Asp Ser His Leu Gly Asp Phe Ile Glu Asp Asp Gly Ala Leu Ser Pro
275 280 285

Ser Asp Asn Ala Ala Tyr Glu Leu Leu Lys Gly Glu Leu Lys Gly Val
290 295 300

Leu Asp Thr Leu Thr Asp Arg Glu Glu Asn Val Leu Arg Leu Arg Phe
305 310 315 320

Gly Leu Asp Asp Gly Arg Gln Arg Thr Leu Glu Asp Val Gly Lys Val
325 330 335

Phe Gly Val Thr Arg Glu Arg Ile Arg Gln Ile Glu Ala Lys Ala Leu

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350

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<211> 1854

<212> DNA

<213> *Alloioococcus otitidis*

<220>

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<222> (1)..(1854)

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gat att gtc gat gtc att ggc caa tac ttg gac tta aac aag tct ggg 96
 Asp Ile Val Asp Val Ile Gly Gln Tyr Leu Asp Leu Asn Lys Ser Gly
 20 25 30

gcc aat tac ttt gcc cac tgc ccc ttc cat gaa gac agc acg cct tct 144
 Ala Asn Tyr Phe Ala His Cys Pro Phe His Glu Asp Ser Thr Pro Ser
 35 40 45

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 Phe Ser Val Asn Arg Asp Lys Gln Ile Tyr Lys Cys Phe Ser Cys Lys
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cga ggt ggc agt gtc ttt agc ttt ata caa gag aag gag gga ctt tcc 240
 Arg Gly Gly Ser Val Phe Ser Phe Ile Gln Glu Lys Glu Gly Leu Ser
 65 70 75 80

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 Phe Pro Glu Ser Val Leu Lys Val Ala Asp Leu Ala Asn Val Asp Leu
 85 90 95

gat ccg gcc tta aaa gaa gct gtc caa ggc caa cct gac aaa gcc gat 336
 Asp Pro Ala Leu Lys Glu Ala Val Gln Gly Gln Pro Asp Lys Ala Asp
 100 105 110

tct ccc tac cga gac ctc tat acc atc cat gac cag gcc aag gac tac 384
 Ser Pro Tyr Arg Asp Leu Tyr Thr Ile His Asp Gln Ala Lys Asp Tyr
 115 120 125

tac cag tat atc ctc tta aag gcc cag gtg gga gaa gtt gct tac gac 432
 Tyr Gln Tyr Ile Leu Leu Lys Ala Gln Val Gly Glu Val Ala Tyr Asp
 130 135 140

tat ctc cag aat cgt ggg att tcc aga gag gtg atg gaa gag ttc gaa Tyr Leu Gln Asn Arg Gly Ile Ser Arg Glu Val Met Glu Glu Phe Glu 145 150 155 160	480
ctg ggt tat tct ccc agc caa agg gag tcg ctc cac ctt tat ttg cag Leu Gly Tyr Ser Pro Ser Gln Arg Glu Ser Leu His Leu Tyr Leu Gln 165 170 175	528
tcc caa gac cag gcg gac ttg aca gat gac tta ctg gaa gaa acc ggc Ser Gln Asp Gln Ala Asp Leu Thr Asp Asp Leu Leu Glu Glu Thr Gly 180 185 190	576
ctt ttt tcc aaa aga gaa gtg gaa agt gat agt ttt aaa gac cgc ttt Leu Phe Ser Lys Arg Glu Val Glu Ser Asp Ser Phe Lys Asp Arg Phe 195 200 205	624
gcc aag cgg atc atc ttc ccc tta aag aac tta caa ggg cag acg gtg Ala Lys Arg Ile Ile Phe Pro Leu Lys Asn Leu Gln Gly Gln Thr Val 210 215 220	672
ggc ttt tcg ggc cgg tat ttc caa gat gag cct aac cag gac ttc cat Gly Phe Ser Gly Arg Tyr Phe Gln Asp Glu Pro Asn Gln Asp Phe His 225 230 235 240	720
cat gcc aag tat tta aac agt cca gaa acc aaa ata ttc aat aaa cgg His Ala Lys Tyr Leu Asn Ser Pro Glu Thr Lys Ile Phe Asn Lys Arg 245 250 255	768
cgg acc ctc ttt aac tac cac cag gcc aag gcc tac att cgt cgg gcc Arg Thr Leu Phe Asn Tyr His Gln Ala Lys Ala Tyr Ile Arg Arg Ala 260 265 270	816
aag gaa gtt gtc tta ttc gaa ggt tac atg gat gtg att gct gct tgg Lys Glu Val Val Leu Phe Glu Gly Tyr Met Asp Val Ile Ala Ala Trp 275 280 285	864
caa gcg ggg gtc aaa aat ggc tta gct tcc atg ggg acc agt ata aca Gln Ala Gly Val Lys Asn Gly Leu Ala Ser Met Gly Thr Ser Ile Thr 290 295 300	912
gct gac caa gtc cag acc atg caa agg att gct gac acc tta gtc ttg Ala Asp Gln Val Gln Thr Met Gln Arg Ile Ala Asp Thr Leu Val Leu 305 310 315 320	960
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gat gac tta agc ttg acc agc aag ctt caa att gaa gtg gtc att ttc Asp Asp Leu Ser Leu Thr Ser Lys Leu Gln Ile Glu Val Val Ile Phe 340 345 350	1056
cct aaa aaa atg gac ccg gat gaa tat att aga gaa aat gga cca gaa Pro Lys Lys Met Asp Pro Asp Glu Tyr Ile Arg Glu Asn Gly Pro Glu 355 360 365	1104
gcc ttt caa aat ctc atc caa cat ggt agg atg act gtc tac caa ttc	1152

Ala	Phe	Gln	Asn	Leu	Ile	Gln	His	Gly	Arg	Met	Thr	Val	Tyr	Gln	Phe	
370						375					380					
tta	aaa	gaa	tac	ttt	aaa	aaa	tcc	tac	aat	cta	gat	aac	gac	tcg	gac	1200
Leu	Lys	Glu	Tyr	Phe	Lys	Lys	Ser	Tyr	Asn	Leu	Asp	Asn	Asp	Ser	Asp	
385					390					395					400	
cgg	ttg	aaa	ttt	atc	caa	acc	atg	acc	aat	aaa	att	ggc	aag	cta	gct	1248
Arg	Leu	Lys	Phe	Ile	Gln	Thr	Met	Thr	Asn	Lys	Ile	Gly	Lys	Leu	Ala	
				405					410					415		
tcc	ccc	ttg	gaa	agg	gaa	gtc	tat	gcc	aag	gat	ttg	gca	gaa	gaa	ttt	1296
Ser	Pro	Leu	Glu	Arg	Glu	Val	Tyr	Ala	Lys	Asp	Leu	Ala	Glu	Glu	Phe	
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aac	ctg	tct	tat	gat	acg	att	ata	agc	caa	gtt	caa	agt	gaa	gcc	act	1344
Asn	Leu	Ser	Tyr	Asp	Thr	Ile	Ile	Ser	Gln	Val	Gln	Ser	Glu	Ala	Thr	
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cta	aac	cag	caa	gag	gct	ttg	aaa	aag	gac	cgg	cat	aag	gaa	ttt	tct	1392
Leu	Asn	Gln	Gln	Glu	Ala	Leu	Lys	Lys	Asp	Arg	His	Lys	Glu	Phe	Ser	
	450					455				460						
caa	gca	aga	gtg	gaa	gtc	aaa	gcc	cca	agt	agt	caa	aag	act	aag	att	1440
Gln	Ala	Arg	Val	Glu	Val	Lys	Ala	Pro	Ser	Ser	Gln	Lys	Thr	Lys	Ile	
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gac	cgg	gcc	cag	gaa	aaa	ctt	tta	aac	cga	ctc	ttt	tac	tat	ccc	caa	1488
Asp	Arg	Ala	Gln	Glu	Lys	Leu	Leu	Asn	Arg	Leu	Phe	Tyr	Tyr	Pro	Gln	
			485					490						495		
gtt	caa	gag	atc	atc	gat	gct	tat	aat	ccg	gac	ttt	gaa	ttt	aaa	acg	1536
Val	Gln	Glu	Ile	Ile	Asp	Ala	Tyr	Asn	Pro	Asp	Phe	Glu	Phe	Lys	Thr	
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gaa	gtc	cac	cag	cgg	att	tac	ctc	ttg	ttt	tta	gaa	tac	agc	cag	gaa	1584
Glu	Val	His	Gln	Arg	Ile	Tyr	Leu	Leu	Phe	Leu	Glu	Tyr	Ser	Gln	Glu	
		515					520					525				
aat	gat	agc	att	gat	tct	ttc	atc	gat	ttt	gtc	aaa	gac	aag	gag	acg	1632
Asn	Asp	Ser	Ile	Asp	Ser	Phe	Ile	Asp	Phe	Val	Lys	Asp	Lys	Glu	Thr	
	530					535				540						
aaa	gag	gtc	ata	tct	gat	ata	atg	tgg	aca	tcc	att	gag	gtc	gaa	ccc	1680
Lys	Glu	Val	Ile	Ser	Asp	Ile	Met	Trp	Thr	Ser	Ile	Glu	Val	Glu	Pro	
545					550					555					560	
tca	gat	gaa	gaa	atc	cta	gac	tac	ttg	gac	tac	att	gac	caa	acc	tac	1728
Ser	Asp	Glu	Glu	Ile	Leu	Asp	Tyr	Leu	Asp	Tyr	Ile	Asp	Gln	Thr	Tyr	
				565				570					575			
ccc	ctg	gag	caa	aaa	cgc	caa	gac	tgc	ttg	gag	gaa	gtc	aaa	gca	gct	1776
Pro	Leu	Glu	Gln	Lys	Arg	Gln	Asp	Cys	Leu	Glu	Glu	Val	Lys	Ala	Ala	
			580					585					590			
aaa	cag	tcc	ggt	aat	aag	aag	cga	gag	ctg	gaa	tta	acc	aat	caa	tta	1824
Lys	Gln	Ser	Gly	Asn	Lys	Lys	Arg	Glu	Leu	Glu	Leu	Thr	Asn	Gln	Leu	

595

600

605

att gaa ata aac cgt atg cta aaa caa taa
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610 615

1854

<210> 64
<211> 617
<212> PRT
<213> *Alloiococcus otitidis*

<400> 64
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Asp Ile Val Asp Val Ile Gly Gln Tyr Leu Asp Leu Asn Lys Ser Gly
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Ala Asn Tyr Phe Ala His Cys Pro Phe His Glu Asp Ser Thr Pro Ser
35 40 45

Phe Ser Val Asn Arg Asp Lys Gln Ile Tyr Lys Cys Phe Ser Cys Lys
50 55 60

Arg Gly Gly Ser Val Phe Ser Phe Ile Gln Glu Lys Glu Gly Leu Ser
65 70 75 80

Phe Pro Glu Ser Val Leu Lys Val Ala Asp Leu Ala Asn Val Asp Leu
85 90 95

Asp Pro Ala Leu Lys Glu Ala Val Gln Gly Gln Pro Asp Lys Ala Asp
100 105 110

Ser Pro Tyr Arg Asp Leu Tyr Thr Ile His Asp Gln Ala Lys Asp Tyr
115 120 125

Tyr Gln Tyr Ile Leu Leu Lys Ala Gln Val Gly Glu Val Ala Tyr Asp
130 135 140

Tyr Leu Gln Asn Arg Gly Ile Ser Arg Glu Val Met Glu Glu Phe Glu
145 150 155 160

Leu Gly Tyr Ser Pro Ser Gln Arg Glu Ser Leu His Leu Tyr Leu Gln
165 170 175

Ser Gln Asp Gln Ala Asp Leu Thr Asp Asp Leu Leu Glu Glu Thr Gly
180 185 190

Leu Phe Ser Lys Arg Glu Val Glu Ser Asp Ser Phe Lys Asp Arg Phe
195 200 205

Ala Lys Arg Ile Ile Phe Pro Leu Lys Asn Leu Gln Gly Gln Thr Val
210 215 220

Gly Phe Ser Gly Arg Tyr Phe Gln Asp Glu Pro Asn Gln Asp Phe His
225 230 235 240

His Ala Lys Tyr Leu Asn Ser Pro Glu Thr Lys Ile Phe Asn Lys Arg
245 250 255

Arg Thr Leu Phe Asn Tyr His Gln Ala Lys Ala Tyr Ile Arg Arg Ala
260 265 270

Lys Glu Val Val Leu Phe Glu Gly Tyr Met Asp Val Ile Ala Ala Trp
275 280 285

Gln Ala Gly Val Lys Asn Gly Leu Ala Ser Met Gly Thr Ser Ile Thr
290 295 300

Ala Asp Gln Val Gln Thr Met Gln Arg Ile Ala Asp Thr Leu Val Leu
305 310 315 320

Ala Phe Asp Gly Asp Glu Ala Gly Leu Glu Ser Ser Lys Lys Ile Leu
325 330 335

Asp Asp Leu Ser Leu Thr Ser Lys Leu Gln Ile Glu Val Val Ile Phe
340 345 350

Pro Lys Lys Met Asp Pro Asp Glu Tyr Ile Arg Glu Asn Gly Pro Glu
355 360 365

Ala Phe Gln Asn Leu Ile Gln His Gly Arg Met Thr Val Tyr Gln Phe
370 375 380

Leu Lys Glu Tyr Phe Lys Lys Ser Tyr Asn Leu Asp Asn Asp Ser Asp
385 390 395 400

Arg Leu Lys Phe Ile Gln Thr Met Thr Asn Lys Ile Gly Lys Leu Ala

405

410

415

Ser Pro Leu Glu Arg Glu Val Tyr Ala Lys Asp Leu Ala Glu Glu Phe
420 425 430

Asn Leu Ser Tyr Asp Thr Ile Ile Ser Gln Val Gln Ser Glu Ala Thr
435 440 445

Leu Asn Gln Gln Glu Ala Leu Lys Lys Asp Arg His Lys Glu Phe Ser
450 455 460

Gln Ala Arg Val Glu Val Lys Ala Pro Ser Ser Gln Lys Thr Lys Ile
465 470 475 480

Asp Arg Ala Gln Glu Lys Leu Leu Asn Arg Leu Phe Tyr Tyr Pro Gln
485 490 495

Val Gln Glu Ile Ile Asp Ala Tyr Asn Pro Asp Phe Glu Phe Lys Thr
500 505 510

Glu Val His Gln Arg Ile Tyr Leu Leu Phe Leu Glu Tyr Ser Gln Glu
515 520 525

Asn Asp Ser Ile Asp Ser Phe Ile Asp Phe Val Lys Asp Lys Glu Thr
530 535 540

Lys Glu Val Ile Ser Asp Ile Met Trp Thr Ser Ile Glu Val Glu Pro
545 550 555 560

Ser Asp Glu Glu Ile Leu Asp Tyr Leu Asp Tyr Ile Asp Gln Thr Tyr
565 570 575

Pro Leu Glu Gln Lys Arg Gln Asp Cys Leu Glu Glu Val Lys Ala Ala
580 585 590

Lys Gln Ser Gly Asn Lys Lys Arg Glu Leu Glu Leu Thr Asn Gln Leu
595 600 605

Ile Glu Ile Asn Arg Met Leu Lys Gln
610 615

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<211> 987

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (55)..(987)

<223>

<400> 65

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ggt ttt gtc acc ctt ctt ggc cgg ccc aat gtg ggc aag tca acc ctg      153
Gly Phe Val Thr Leu Leu Gly Arg Pro Asn Val Gly Lys Ser Thr Leu
      20                               25                               30

ctc aac caa ata tta ggc cag aag att acc att atc agt gac aaa ccc      201
Leu Asn Gln Ile Leu Gly Gln Lys Ile Thr Ile Ile Ser Asp Lys Pro
      35                               40                               45

caa aca acc cgg aat aaa atc cag ggt att tac acc gac caa gcg ggg      249
Gln Thr Thr Arg Asn Lys Ile Gln Gly Ile Tyr Thr Asp Gln Ala Gly
      50                               55                               60                               65

caa att gtc ttt atc gac aca cct ggt ata cat aaa ccc aag cac cgc      297
Gln Ile Val Phe Ile Asp Thr Pro Gly Ile His Lys Pro Lys His Arg
      70                               75                               80

ctg ggc cgg ttt atg gtg gat tcg gct atg tcg acc atc aat gag gtg      345
Leu Gly Arg Phe Met Val Asp Ser Ala Met Ser Thr Ile Asn Glu Val
      85                               90                               95

gac ctg gtc tta ttt gtg gtc aat gtc agg gaa aag att ggc ccg ggg      393
Asp Leu Val Leu Phe Val Val Asn Val Arg Glu Lys Ile Gly Pro Gly
      100                               105                               110

gac cgg ttc att atc gac aag ttg cga acc atc gat acg cca gtt ttt      441
Asp Arg Phe Ile Ile Asp Lys Leu Arg Thr Ile Asp Thr Pro Val Phe
      115                               120                               125

tta att att aac cag att gac cag gtc gat cca aca gac ctc cta ccg      489
Leu Ile Ile Asn Gln Ile Asp Gln Val Asp Pro Thr Asp Leu Leu Pro
      130                               135                               140                               145

gtt att agc gac tac caa gag gaa ttc gac ttt gcc gaa gtg gtt cca      537
Val Ile Ser Asp Tyr Gln Glu Glu Phe Asp Phe Ala Glu Val Val Pro
      150                               155                               160

act tca ggc ttg gaa ggg gaa aat atc cag gag ctc att caa acc atc      585
Thr Ser Gly Leu Glu Gly Glu Asn Ile Gln Glu Leu Ile Gln Thr Ile
      165                               170                               175

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aag tct tac cta cct gtt gga ccc caa ttt tac ccg gac gac cag gtc 633
Lys Ser Tyr Leu Pro Val Gly Pro Gln Phe Tyr Pro Asp Asp Gln Val
180 185 190

tcg gac cac ccc gaa tac ttt att att tca gaa ctc atc cgg gag aag 681
Ser Asp His Pro Glu Tyr Phe Ile Ile Ser Glu Leu Ile Arg Glu Lys
195 200 205

gtt tta gac ttg gct aga gaa gag att cct cat tca gta gca gta gta 729
Val Leu Asp Leu Ala Arg Glu Glu Ile Pro His Ser Val Ala Val Val
210 215 220 225

act gag aag gta gac cga aac caa gat ggt aaa gtc caa acc tat gcc 777
Thr Glu Lys Val Asp Arg Asn Gln Asp Gly Lys Val Gln Thr Tyr Ala
230 235 240

acc att att gtc gaa cgc aag agc caa aag ggg att att atc ggc aag 825
Thr Ile Ile Val Glu Arg Lys Ser Gln Lys Gly Ile Ile Ile Gly Lys
245 250 255

caa ggg tcc atg att aaa aaa att ggt agc cta gct cgg cga gat att 873
Gln Gly Ser Met Ile Lys Lys Ile Gly Ser Leu Ala Arg Arg Asp Ile
260 265 270

gag aaa cta ctg gga gat aag att tac ttg gaa ctc tgg gtt aaa gtc 921
Glu Lys Leu Leu Gly Asp Lys Ile Tyr Leu Glu Leu Trp Val Lys Val
275 280 285

caa aga gac tgg cgg gac aag ccc agt cgc tta gaa gac ttt ggc tac 969
Gln Arg Asp Trp Arg Asp Lys Pro Ser Arg Leu Glu Asp Phe Gly Tyr
290 295 300 305

aat gaa gac aac tat tag 987
Asn Glu Asp Asn Tyr
310

<210> 66

<211> 310

<212> PRT

<213> *Alloiococcus otitidis*

<400> 66

Met Glu Asn Asn Glu Asn Asn Glu Asn Lys Asp Ser Lys Thr Phe Lys
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Ser Gly Phe Val Thr Leu Leu Gly Arg Pro Asn Val Gly Lys Ser Thr
20 25 30

Leu Leu Asn Gln Ile Leu Gly Gln Lys Ile Thr Ile Ile Ser Asp Lys
35 40 45

Pro Gln Thr Thr Arg Asn Lys Ile Gln Gly Ile Tyr Thr Asp Gln Ala
50 55 60

Gly Gln Ile Val Phe Ile Asp Thr Pro Gly Ile His Lys Pro Lys His
65 70 75 80

Arg Leu Gly Arg Phe Met Val Asp Ser Ala Met Ser Thr Ile Asn Glu
85 90 95

Val Asp Leu Val Leu Phe Val Val Asn Val Arg Glu Lys Ile Gly Pro
100 105 110

Gly Asp Arg Phe Ile Ile Asp Lys Leu Arg Thr Ile Asp Thr Pro Val
115 120 125

Phe Leu Ile Ile Asn Gln Ile Asp Gln Val Asp Pro Thr Asp Leu Leu
130 135 140

Pro Val Ile Ser Asp Tyr Gln Glu Glu Phe Asp Phe Ala Glu Val Val
145 150 155 160

Pro Thr Ser Gly Leu Glu Gly Glu Asn Ile Gln Glu Leu Ile Gln Thr
165 170 175

Ile Lys Ser Tyr Leu Pro Val Gly Pro Gln Phe Tyr Pro Asp Asp Gln
180 185 190

Val Ser Asp His Pro Glu Tyr Phe Ile Ile Ser Glu Leu Ile Arg Glu
195 200 205

Lys Val Leu Asp Leu Ala Arg Glu Glu Ile Pro His Ser Val Ala Val
210 215 220

Val Thr Glu Lys Val Asp Arg Asn Gln Asp Gly Lys Val Gln Thr Tyr
225 230 235 240

Ala Thr Ile Ile Val Glu Arg Lys Ser Gln Lys Gly Ile Ile Ile Gly
245 250 255

Lys Gln Gly Ser Met Ile Lys Lys Ile Gly Ser Leu Ala Arg Arg Asp
260 265 270

Ile Glu Lys Leu Leu Gly Asp Lys Ile Tyr Leu Glu Leu Trp Val Lys
275 280 285

Val Gln Arg Asp Trp Arg Asp Lys Pro Ser Arg Leu Glu Asp Phe Gly
 290 295 300

Tyr Asn Glu Asp Asn Tyr
 305 310

<210> 67
 <211> 1557
 <212> DNA
 <213> *Alloiococcus otitidis*

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 <223>

<400> 67
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 Met Ile Ser Ser
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ttc tat tta gta gga gtc ttg aga ttg agt agt gaa aat aaa tta acc 105
 Phe Tyr Leu Val Gly Val Leu Arg Leu Ser Ser Glu Asn Lys Leu Thr
 5 10 15 20

ttc aaa cac ttc ctt gca aac cag ttg acc aaa cga gac aat tta caa 153
 Phe Lys His Phe Leu Ala Asn Gln Leu Thr Lys Arg Asp Asn Leu Gln
 25 30 35

atc ccc cgt tgg caa att ttt gcc gtt tta ttt aca gga gcc gtg att 201
 Ile Pro Arg Trp Gln Ile Phe Ala Val Leu Phe Thr Gly Ala Val Ile
 40 45 50

gtg gtt ctc aac caa acg gcc atg tct acc gcc ttg cct aat atg att 249
 Val Val Leu Asn Gln Thr Ala Met Ser Thr Ala Leu Pro Asn Met Ile
 55 60 65

gaa agt ttg ggc att gac cct agc cta ggc cag tgg att gtc tcg ggt 297
 Glu Ser Leu Gly Ile Asp Pro Ser Leu Gly Gln Trp Ile Val Ser Gly
 70 75 80

tat acc ttg gtc aaa ggg att atg gtc ccc ata acc gcc ttt gcc atg 345
 Tyr Thr Leu Val Lys Gly Ile Met Val Pro Ile Thr Ala Phe Ala Met
 85 90 95 100

acc aag tac cgg aca cgg aac ttt ttt att tta atg ttg gcc ctc ttc 393
 Thr Lys Tyr Arg Thr Arg Asn Phe Phe Ile Leu Met Leu Ala Leu Phe
 105 110 115

tgt acc ggt agt ttt ttg act ggt ctg ggc ttt aat ttt ccg gtt gtg 441
 Cys Thr Gly Ser Phe Leu Thr Gly Leu Gly Phe Asn Phe Pro Val Val
 120 125 130

gtc atg ggg aca gtc atc cag ggt ata gcg gct ggg atg atc atc ccc 489

Val	Met	Gly	Thr	Val	Ile	Gln	Gly	Ile	Ala	Ala	Gly	Met	Ile	Ile	Pro	
		135					140					145				
ttg	atg	cag	acc	gtc	ctc	ttg	acc	ttg	atg	ccg	gtt	gaa	agc	cga	ggc	537
Leu	Met	Gln	Thr	Val	Leu	Leu	Thr	Leu	Met	Pro	Val	Glu	Ser	Arg	Gly	
	150					155					160					
act	gct	atg	ggg	gta	atg	agt	ggg	gtt	att	ggg	att	ggg	cca	gca	ctg	585
Thr	Ala	Met	Gly	Val	Met	Ser	Gly	Val	Ile	Gly	Ile	Gly	Pro	Ala	Leu	
165					170				175						180	
ggg	ccc	ctt	gtc	ggg	ggg	gtc	att	gtt	gat	gct	ttc	acc	tgg	gaa	att	633
Gly	Pro	Leu	Val	Gly	Gly	Val	Ile	Val	Asp	Ala	Phe	Thr	Trp	Glu	Ile	
				185					190					195		
tta	ttc	tac	atc	tgg	gcc	tta	atc	acc	ctt	tta	ttg	gtt	cct	tta	act	681
Leu	Phe	Tyr	Ile	Trp	Ala	Leu	Ile	Thr	Leu	Leu	Leu	Val	Pro	Leu	Thr	
			200					205					210			
tgg	ctg	gtc	tta	ccc	gat	gta	ttg	cca	aat	gca	gat	tta	acc	att	aat	729
Trp	Leu	Val	Leu	Pro	Asp	Val	Leu	Pro	Asn	Ala	Asp	Leu	Thr	Ile	Asn	
		215					220					225				
tgg	gcc	aat	atc	cgg	gac	tcc	ctc	att	ggg	ttt	ggc	ctc	ctc	ctc	ttt	777
Trp	Ala	Asn	Ile	Arg	Asp	Ser	Leu	Ile	Gly	Phe	Gly	Leu	Leu	Leu	Phe	
	230					235					240					
agc	ttg	tca	gtc	ttt	ggg	tct	tcc	ggg	ttt	tct	tcc	gtc	att	gcc	tgg	825
Ser	Leu	Ser	Val	Phe	Gly	Ser	Ser	Gly	Phe	Ser	Ser	Val	Ile	Ala	Trp	
245					250				255						260	
gtc	agc	ttg	ctt	atc	ggg	tta	gtc	ttt	gtc	gcc	aag	ttt	atc	cac	ttc	873
Val	Ser	Leu	Leu	Ile	Gly	Leu	Val	Phe	Val	Ala	Lys	Phe	Ile	His	Phe	
				265				270						275		
aac	ctc	aag	gca	gac	caa	cca	atc	tta	aat	ctt	aga	ctc	ttt	aaa	aaa	921
Asn	Leu	Lys	Ala	Asp	Gln	Pro	Ile	Leu	Asn	Leu	Arg	Leu	Phe	Lys	Lys	
			280					285					290			
acc	tat	tac	cgt	cgg	gct	gtc	ttg	gta	gcc	acc	ttg	ggg	att	gtc	att	969
Thr	Tyr	Tyr	Arg	Arg	Ala	Val	Leu	Val	Ala	Thr	Leu	Gly	Ile	Val	Ile	
		295					300					305				
att	tct	tgt	cta	tcc	aac	att	atc	cct	att	tat	gtt	caa	act	gtt	agg	1017
Ile	Ser	Cys	Leu	Ser	Asn	Ile	Ile	Pro	Ile	Tyr	Val	Gln	Thr	Val	Arg	
	310					315					320					
ggc	ttg	ggg	gct	tcc	ata	gca	ggc	tta	atc	tta	atg	cca	gct	ggg	atc	1065
Gly	Leu	Gly	Ala	Ser	Ile	Ala	Gly	Leu	Ile	Leu	Met	Pro	Ala	Gly	Ile	
325					330				335						340	
atc	aaa	acc	atc	tta	gct	cct	atc	tca	ggc	aaa	ctt	tat	gac	aag	gtt	1113
Ile	Lys	Thr	Ile	Leu	Ala	Pro	Ile	Ser	Gly	Lys	Leu	Tyr	Asp	Lys	Val	
				345				350						355		
gga	gtg	gct	cgg	att	ggc	ctt	atc	ggg	ggg	atc	tta	ctt	tta	gtt	ggg	1161
Gly	Val	Ala	Arg	Ile	Gly	Leu	Ile	Gly	Gly	Ile	Leu	Leu	Leu	Val	Gly	

360	365	370	
tcc tta tta cta gtt acc ctc aat gaa gct agc tcc ctt tac tta ctg			1209
Ser Leu Leu Leu Val Thr Leu Asn Glu Ala Ser Ser Leu Tyr Leu Leu			
375	380	385	
atg att tac tac ggc atc tta tca gcc ggt ttt ggc ttg ttt aat atc			1257
Met Ile Tyr Tyr Gly Ile Leu Ser Ala Gly Phe Gly Leu Phe Asn Ile			
390	395	400	
cct att acc act gct ggc atg aat att atg gcc aag gaa gat atg gga			1305
Pro Ile Thr Thr Ala Gly Met Asn Ile Met Ala Lys Glu Asp Met Gly			
405	410	415	420
cat gcg act tca gcc cgg caa acg gtc cgg caa atc tct tca agt ttt			1353
His Ala Thr Ser Ala Arg Gln Thr Val Arg Gln Ile Ser Ser Ser Phe			
425	430	435	
gcc gtt tcc ctc tcc ttt atc atc atg acc ctg gtg act att gcc act			1401
Ala Val Ser Leu Ser Phe Ile Ile Met Thr Leu Val Thr Ile Ala Thr			
440	445	450	
tcc ggc caa tcg gtg ggg gtt ttc caa gat ggc ggt ccg aca gac tta			1449
Ser Gly Gln Ser Val Gly Val Phe Gln Asp Gly Gly Pro Thr Asp Leu			
455	460	465	
aat atg gca gga gtc cga ggc gcc ttt atc ttg gtg gct ata ttt tca			1497
Asn Met Ala Gly Val Arg Gly Ala Phe Ile Leu Val Ala Ile Phe Ser			
470	475	480	
atc cta gcc atg atc ttg atc ttc ttt tta aaa gac cct aaa gaa aaa			1545
Ile Leu Ala Met Ile Leu Ile Phe Phe Leu Lys Asp Pro Lys Glu Lys			
485	490	495	500
cca gac caa tag			1557
Pro Asp Gln			

<210> 68

<211> 503

<212> PRT

<213> *Alloiococcus otitidis*

<400> 68

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Asn	Lys	Leu	Thr	Phe	Lys	His	Phe	Leu	Ala	Asn	Gln	Leu	Thr	Lys	Arg
			20					25					30		

Asp	Asn	Leu	Gln	Ile	Pro	Arg	Trp	Gln	Ile	Phe	Ala	Val	Leu	Phe	Thr
		35					40					45			

Gly	Ala	Val	Ile	Val	Val	Leu	Asn	Gln	Thr	Ala	Met	Ser	Thr	Ala	Leu
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

50

55

60

Pro Asn Met Ile Glu Ser Leu Gly Ile Asp Pro Ser Leu Gly Gln Trp
 65 70 75 80

Ile Val Ser Gly Tyr Thr Leu Val Lys Gly Ile Met Val Pro Ile Thr
 85 90 95

Ala Phe Ala Met Thr Lys Tyr Arg Thr Arg Asn Phe Phe Ile Leu Met
 100 105 110

Leu Ala Leu Phe Cys Thr Gly Ser Phe Leu Thr Gly Leu Gly Phe Asn
 115 120 125

Phe Pro Val Val Val Met Gly Thr Val Ile Gln Gly Ile Ala Ala Gly
 130 135 140

Met Ile Ile Pro Leu Met Gln Thr Val Leu Leu Thr Leu Met Pro Val
 145 150 155 160

Glu Ser Arg Gly Thr Ala Met Gly Val Met Ser Gly Val Ile Gly Ile
 165 170 175

Gly Pro Ala Leu Gly Pro Leu Val Gly Gly Val Ile Val Asp Ala Phe
 180 185 190

Thr Trp Glu Ile Leu Phe Tyr Ile Trp Ala Leu Ile Thr Leu Leu Leu
 195 200 205

Val Pro Leu Thr Trp Leu Val Leu Pro Asp Val Leu Pro Asn Ala Asp
 210 215 220

Leu Thr Ile Asn Trp Ala Asn Ile Arg Asp Ser Leu Ile Gly Phe Gly
 225 230 235 240

Leu Leu Leu Phe Ser Leu Ser Val Phe Gly Ser Ser Gly Phe Ser Ser
 245 250 255

Val Ile Ala Trp Val Ser Leu Leu Ile Gly Leu Val Phe Val Ala Lys
 260 265 270

Phe Ile His Phe Asn Leu Lys Ala Asp Gln Pro Ile Leu Asn Leu Arg
 275 280 285

Leu Phe Lys Lys Thr Tyr Tyr Arg Arg Ala Val Leu Val Ala Thr Leu
290 295 300

Gly Ile Val Ile Ile Ser Cys Leu Ser Asn Ile Ile Pro Ile Tyr Val
305 310 315 320

Gln Thr Val Arg Gly Leu Gly Ala Ser Ile Ala Gly Leu Ile Leu Met
325 330 335

Pro Ala Gly Ile Ile Lys Thr Ile Leu Ala Pro Ile Ser Gly Lys Leu
340 345 350

Tyr Asp Lys Val Gly Val Ala Arg Ile Gly Leu Ile Gly Gly Ile Leu
355 360 365

Leu Leu Val Gly Ser Leu Leu Leu Val Thr Leu Asn Glu Ala Ser Ser
370 375 380

Leu Tyr Leu Leu Met Ile Tyr Tyr Gly Ile Leu Ser Ala Gly Phe Gly
385 390 395 400

Leu Phe Asn Ile Pro Ile Thr Thr Ala Gly Met Asn Ile Met Ala Lys
405 410 415

Glu Asp Met Gly His Ala Thr Ser Ala Arg Gln Thr Val Arg Gln Ile
420 425 430

Ser Ser Ser Phe Ala Val Ser Leu Ser Phe Ile Ile Met Thr Leu Val
435 440 445

Thr Ile Ala Thr Ser Gly Gln Ser Val Gly Val Phe Gln Asp Gly Gly
450 455 460

Pro Thr Asp Leu Asn Met Ala Gly Val Arg Gly Ala Phe Ile Leu Val
465 470 475 480

Ala Ile Phe Ser Ile Leu Ala Met Ile Leu Ile Phe Phe Leu Lys Asp
485 490 495

Pro Lys Glu Lys Pro Asp Gln
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<210> 69
 <211> 4392
 <212> DNA
 <213> *Alloiococcus otitidis*

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 <222> (58)..(4392)
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<400> 69
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 Met Ser Leu Asn Gln Lys Glu Met Tyr Gln Val Leu Met Gln Gln Val
 1 5 10 15

cac tta gaa gaa cac cta caa gac cga ccc ctt ctt aaa gcc ggc agt 153
 His Leu Glu Glu His Leu Gln Asp Arg Pro Leu Leu Lys Ala Gly Ser
 20 25 30

ttg aag caa att gtt gtt tac aag gct caa caa gcc tgg gac ctg acc 201
 Leu Lys Gln Ile Val Val Tyr Lys Ala Gln Gln Ala Trp Asp Leu Thr
 35 40 45

ctc caa ttt cct cag atc ctc cct ttt aag gac ttc caa gtt ttg gag 249
 Leu Gln Phe Pro Gln Ile Leu Pro Phe Lys Asp Phe Gln Val Leu Glu
 50 55 60

tct gcc ctc ttg cag cat atc cca gaa gtc aac cag atc cat tta agg 297
 Ser Ala Leu Leu Gln His Ile Pro Glu Val Asn Gln Ile His Leu Arg
 65 70 75 80

gtt gat gcc caa gat gac agt ttt gac cag gac ctc ctc cag gac tat 345
 Val Asp Ala Gln Asp Asp Ser Phe Asp Gln Asp Leu Leu Gln Asp Tyr
 85 90 95

tgg cct aag gcg gtg aag ttt agc gga gtc gat tct ccc ctt tgc aat 393
 Trp Pro Lys Ala Val Lys Phe Ser Gly Val Asp Ser Pro Leu Cys Asn
 100 105 110

gac tta cta gac aag acc ctc cct tat cta gat ggg aag caa gtt tac 441
 Asp Leu Leu Asp Lys Thr Leu Pro Tyr Leu Asp Gly Lys Gln Val Tyr
 115 120 125

ttt gac ctg gac cat gaa gtg acc cgg gac aag ttt gac cat gac ttc 489
 Phe Asp Leu Asp His Glu Val Thr Arg Asp Lys Phe Asp His Asp Phe
 130 135 140

cta cct cgg atc caa gct ggc tac cag caa gtg ggc ttt ccc aac cac 537
 Leu Pro Arg Ile Gln Ala Gly Tyr Gln Gln Val Gly Phe Pro Asn His
 145 150 155 160

ttt aaa atc aag gct agg gtc gat gcc cag aaa aat tca gat caa att 585

Phe Lys Ile Lys Ala Arg Val Asp Ala Gln Lys Asn Ser Asp Gln Ile	
165 170 175	
gcc gcc ttc cgt aaa gaa aaa gaa gaa aaa gac cag gcc ttg tct caa	633
Ala Ala Phe Arg Lys Glu Lys Glu Glu Lys Asp Gln Ala Leu Ser Gln	
180 185 190	
gag cta acc aac caa ttt atc aag gcc agc caa aag aaa gaa gaa ggg	681
Glu Leu Thr Asn Gln Phe Ile Lys Ala Ser Gln Lys Lys Glu Glu Gly	
195 200 205	
gga tcc aaa gcc aag tcg gag gcc ttg aag atg ggc cgg gcc atc cct	729
Gly Ser Lys Ala Lys Ser Glu Ala Leu Lys Met Gly Arg Ala Ile Pro	
210 215 220	
gac cac gaa acg att acc cag atg gtt gat gtg gaa gaa gaa gag agc	777
Asp His Glu Thr Ile Thr Gln Met Val Asp Val Glu Glu Glu Glu Ser	
225 230 235 240	
cgt ctg acc ttt gaa gga tac gtt ttt gat gtg gaa atc aaa tcc ctc	825
Arg Leu Thr Phe Glu Gly Tyr Val Phe Asp Val Glu Ile Lys Ser Leu	
245 250 255	
cgg tca gat aga aag ctc ctt ctc ttt aaa atg acc gac tat agc tct	873
Arg Ser Asp Arg Lys Leu Leu Leu Phe Lys Met Thr Asp Tyr Ser Ser	
260 265 270	
tcc ttc cta ttc aaa aaa ttc tct aat aat tct tct gac gaa gcc cta	921
Ser Phe Leu Phe Lys Lys Phe Ser Asn Asn Ser Ser Asp Glu Ala Leu	
275 280 285	
ttt gac caa gtc caa gag gga atg tgg ctc aag gtt aga ggc agt gtt	969
Phe Asp Gln Val Gln Glu Gly Met Trp Leu Lys Val Arg Gly Ser Val	
290 295 300	
caa gaa gat acc ttt gtc aaa gac cta gtt gtc atg gcc caa gac atc	1017
Gln Glu Asp Thr Phe Val Lys Asp Leu Val Val Met Ala Gln Asp Ile	
305 310 315 320	
caa gag gtc aaa aaa gaa ccc cgg cgg gac ctg gct aag gaa ggg gag	1065
Gln Glu Val Lys Lys Glu Pro Arg Arg Asp Leu Ala Lys Glu Gly Glu	
325 330 335	
aag agg gtg gaa ctt cat gcc cat acc acc atg agt cag atg gac ggt	1113
Lys Arg Val Glu Leu His Ala His Thr Thr Met Ser Gln Met Asp Gly	
340 345 350	
ttg gtg ccg gcc aag gat ttg gtc aag caa gca gcc gct ttt gac caa	1161
Leu Val Pro Ala Lys Asp Leu Val Lys Gln Ala Ala Ala Phe Asp Gln	
355 360 365	
ccg gct att gcc atc act gat cat gct gta gtc caa tcc ttc cca gag	1209
Pro Ala Ile Ala Ile Thr Asp His Ala Val Val Gln Ser Phe Pro Glu	
370 375 380	
gcc cat tat gct ggc tta gac act ggt gtt aaa att ctt tac ggt gtg	1257
Ala His Tyr Ala Gly Leu Asp Thr Gly Val Lys Ile Leu Tyr Gly Val	

385	390	395	400	
gaa gcc aat ttg gtt agt gat ggc gaa ttg gta gca tac aat ccg gcc				1305
Glu Ala Asn Leu Val Ser Asp Gly Glu Leu Val Ala Tyr Asn Pro Ala				
	405	410	415	
gat ata aag ctg gaa gag gca act tat gtg gtc ttc gac gtg gaa aca				1353
Asp Ile Lys Leu Glu Glu Ala Thr Tyr Val Val Phe Asp Val Glu Thr				
	420	425	430	
acc gga cta tcg gct cgt tat gac caa atc att gaa ttg gcc gct gtg				1401
Thr Gly Leu Ser Ala Arg Tyr Asp Gln Ile Ile Glu Leu Ala Ala Val				
	435	440	445	
aag atg gaa aat ggg gaa atc gtt tct gaa ttc caa gaa ttt att gac				1449
Lys Met Glu Asn Gly Glu Ile Val Ser Glu Phe Gln Glu Phe Ile Asp				
	450	455	460	
cca ggc cag ccc ttg tct gag act acg acc aat ttg acc ggg atc acc				1497
Pro Gly Gln Pro Leu Ser Glu Thr Thr Thr Asn Leu Thr Gly Ile Thr				
	465	470	475	480
gat gac atg gtc caa gga tcc aaa agt gaa gac gaa gtc ctc cat gcc				1545
Asp Asp Met Val Gln Gly Ser Lys Ser Glu Asp Glu Val Leu His Ala				
	485	490	495	
ttt caa gcc ttt tca gaa ggc act gtc ttg gtc gcc cat aac gct tcc				1593
Phe Gln Ala Phe Ser Glu Gly Thr Val Leu Val Ala His Asn Ala Ser				
	500	505	510	
ttt gac atg ggc ttt atc aat acg gcc tac caa cga cat ggc cta gga				1641
Phe Asp Met Gly Phe Ile Asn Thr Ala Tyr Gln Arg His Gly Leu Gly				
	515	520	525	
caa gct gac cag cct gtg att gat acc ttg gaa ttg tcc cgc atg ctc				1689
Gln Ala Asp Gln Pro Val Ile Asp Thr Leu Glu Leu Ser Arg Met Leu				
	530	535	540	
cac cca aac ttg aaa agc cac cgg tta aac act ctg gct aag cgg tat				1737
His Pro Asn Leu Lys Ser His Arg Leu Asn Thr Leu Ala Lys Arg Tyr				
	545	550	555	560
gac gtg gcc tta gaa cac cac cac cgg gcc atc tat gac tcg gag tca				1785
Asp Val Ala Leu Glu His His His Arg Ala Ile Tyr Asp Ser Glu Ser				
	565	570	575	
acg gct aaa ctc ttg tgg atc ttc tta aaa gaa gcc aaa gac caa tat				1833
Thr Ala Lys Leu Leu Trp Ile Phe Leu Lys Glu Ala Lys Asp Gln Tyr				
	580	585	590	
gac atg act agc cac caa gac ttg aat agc cag gtg ggg gaa ggc gag				1881
Asp Met Thr Ser His Gln Asp Leu Asn Ser Gln Val Gly Glu Gly Glu				
	595	600	605	
gct tac aag cag gcc cgg cca acc cat gcc agt att ttg gtc aag aat				1929
Ala Tyr Lys Gln Ala Arg Pro Thr His Ala Ser Ile Leu Val Lys Asn				
	610	615	620	

caa aaa ggc ttg aaa aac ctc ttt aaa att gtc tcc cac gcc cat gtc Gln Lys Gly Leu Lys Asn Leu Phe Lys Ile Val Ser His Ala His Val 625 630 635 640	1977
aac tac ttc tac cgg gtt ccc cgt ata cct aag tct atc ttg agc aag Asn Tyr Phe Tyr Arg Val Pro Arg Ile Pro Lys Ser Ile Leu Ser Lys 645 650 655	2025
tac cgg gaa ggc ctt ttg gtt ggg tct ggt tgc gga cag gga gag ctc Tyr Arg Glu Gly Leu Leu Val Gly Ser Gly Cys Gly Gln Gly Glu Leu 660 665 670	2073
ttt gag gct att atg caa aag ggc tat gac gaa gcc ttg gca gtt gcc Phe Glu Ala Ile Met Gln Lys Gly Tyr Asp Glu Ala Leu Ala Val Ala 675 680 685	2121
cag gac tat gat tat att gaa gtt atg ccc aag tca gcc tat att gac Gln Asp Tyr Asp Tyr Ile Glu Val Met Pro Lys Ser Ala Tyr Ile Asp 690 695 700	2169
ctc ttg gac cgg gac tta atc aag gat gag gca acc ctt gaa gaa atg Leu Leu Asp Arg Asp Leu Ile Lys Asp Glu Ala Thr Leu Glu Glu Met 705 710 715 720	2217
att gaa aac ctg gtt aaa ata ggc cat gaa ctt gat ata ccc gtg gta Ile Glu Asn Leu Val Lys Ile Gly His Glu Leu Asp Ile Pro Val Val 725 730 735	2265
gct aca ggg aat gtc cac tac cta aac cca gaa gat gcc gtt tta cgg Ala Thr Gly Asn Val His Tyr Leu Asn Pro Glu Asp Ala Val Leu Arg 740 745 750	2313
gat atc ctc ctg gaa act gcc aaa aag gga gcc ttc tcc aaa gcc cgg Asp Ile Leu Leu Glu Thr Ala Lys Lys Gly Ala Phe Ser Lys Ala Arg 755 760 765	2361
aac cca gaa gtc cac ttt aga aca aca gat gaa atg tta gaa gag ttt Asn Pro Glu Val His Phe Arg Thr Thr Asp Glu Met Leu Glu Glu Phe 770 775 780	2409
tcc ttc cta ggc cag gac cag gct tat gag att gtg gtc acc aac acc Ser Phe Leu Gly Gln Asp Gln Ala Tyr Glu Ile Val Val Thr Asn Thr 785 790 795 800	2457
caa aaa att gct gat tct atc gaa tca atc tct cct gtc aag gaa ggc Gln Lys Ile Ala Asp Ser Ile Glu Ser Ile Ser Pro Val Lys Glu Gly 805 810 815	2505
ctc tat gcc ccg aaa atg gaa ggg tcg gac caa gag ata cgt cag atg Leu Tyr Ala Pro Lys Met Glu Gly Ser Asp Gln Glu Ile Arg Gln Met 820 825 830	2553
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gta gag gaa agg ctc gaa aaa gag ttg aag agt att att gac aac aat	2649
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ttc tct gtc att tac tta att tcc cag aaa ttg gtc aaa aaa agt gtt	2697
Phe Ser Val Ile Tyr Leu Ile Ser Gln Lys Leu Val Lys Lys Ser Val	
865 870 875 880	
gaa gat ggc tat ttg gtt ggt tcc agg ggg tcg gtt ggg tca agc ttt	2745
Glu Asp Gly Tyr Leu Val Gly Ser Arg Gly Ser Val Gly Ser Ser Phe	
885 890 895	
gtg gcc acc atg acc ggg atc aca gaa gtc aac cca cta ccg ccc cac	2793
Val Ala Thr Met Thr Gly Ile Thr Glu Val Asn Pro Leu Pro Pro His	
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tac cgc tgt cct aac tgc cag cac acc gaa ttc ttc aca aat ggg gaa	2841
Tyr Arg Cys Pro Asn Cys Gln His Thr Glu Phe Phe Thr Asn Gly Glu	
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gtg ggg tcc ggc ttt gac tta gag gcc aaa aaa tgt ccg gaa tgt caa	2889
Val Gly Ser Gly Phe Asp Leu Glu Ala Lys Lys Cys Pro Glu Cys Gln	
930 935 940	
agc cta atg gaa tca gac ggc cac gac att ccc ttc gaa acc ttc ctt	2937
Ser Leu Met Glu Ser Asp Gly His Asp Ile Pro Phe Glu Thr Phe Leu	
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965 970 975	
gaa tac cag gcc aag gcc cac aac tat acc aag gtt ttg ttt gga gaa	3033
Glu Tyr Gln Ala Lys Ala His Asn Tyr Thr Lys Val Leu Phe Gly Glu	
980 985 990	
gac cat gtc tac cgg gca ggg acc atc acg acg att gct gac aag acg	3081
Asp His Val Tyr Arg Ala Gly Thr Ile Thr Thr Ile Ala Asp Lys Thr	
995 1000 1005	
gcc ttt ggt ttt gtc aag ggt tat gaa agg gac aag cag ata aac tac	3129
Ala Phe Gly Phe Val Lys Gly Tyr Glu Arg Asp Lys Gln Ile Asn Tyr	
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cgg tcg gct gaa gtg gac cgg ctg tca gat ggt tta acc gga gtg aga	3177
Arg Ser Ala Glu Val Asp Arg Leu Ser Asp Gly Leu Thr Gly Val Arg	
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cgg tca acc ggc cag cac cca gga ggg att atc gtc ata ccg gat gac	3225
Arg Ser Thr Gly Gln His Pro Gly Gly Ile Ile Val Ile Pro Asp Asp	
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atg gat gtg ttt gat ttc acc ccc atc cag tac ccg gct gac gac cag	3273
Met Asp Val Phe Asp Phe Thr Pro Ile Gln Tyr Pro Ala Asp Asp Gln	
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acg gct gag tgg caa act acc cac ttt gac ttc cac tcc atc gac gaa	3321

Thr Ala Glu Trp Gln Thr Thr His Phe Asp Phe His Ser Ile Asp Glu 1075 1080 1085	
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cga aaa ctc cag gac ttg tcc ggc ttt gac cct caa gaa ata ccg gta Arg Lys Leu Gln Asp Leu Ser Gly Phe Asp Pro Gln Glu Ile Pro Val 1105 1110 1115 1120	3417
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tct acc ttt gct gaa ctc ttg cag atc tca ggc ctc tcc cac ggg aca Ser Thr Phe Ala Glu Leu Leu Gln Ile Ser Gly Leu Ser His Gly Thr 1170 1175 1180	3609
gat gtt tgg ctg ggc aat gct gaa gaa tta att cgc aac cac aac att Asp Val Trp Leu Gly Asn Ala Glu Glu Leu Ile Arg Asn His Asn Ile 1185 1190 1195 1200	3657
ccc ttg tcc gag gtg atc ggc tgc cgg gat gat atc atg gtc tac ctt Pro Leu Ser Glu Val Ile Gly Cys Arg Asp Asp Ile Met Val Tyr Leu 1205 1210 1215	3705
caa cac caa ggt ctt gaa gac agc ctg gcc ttt aag att atg gaa ttt Gln His Gln Gly Leu Glu Asp Ser Leu Ala Phe Lys Ile Met Glu Phe 1220 1225 1230	3753
gtt cgt aag ggt cgg ggc ttg caa gat gac tgg att gct acc atg aaa Val Arg Lys Gly Arg Gly Leu Gln Asp Asp Trp Ile Ala Thr Met Lys 1235 1240 1245	3801
gaa aat gat gtt cct gat tgg tat att gaa tcc tgc aaa aaa atc aag Glu Asn Asp Val Pro Asp Trp Tyr Ile Glu Ser Cys Lys Lys Ile Lys 1250 1255 1260	3849
tac atg ttc cct aaa gcc cac gca gct gcc tat gtc ttg atg gcc ctt Tyr Met Phe Pro Lys Ala His Ala Ala Tyr Val Leu Met Ala Leu 1265 1270 1275 1280	3897
agg gta gct tac ttt aaa gtc cac tac ccc ctt tac tac tac gct gcc Arg Val Ala Tyr Phe Lys Val His Tyr Pro Leu Tyr Tyr Tyr Ala Ala 1285 1290 1295	3945
tac ttt tcc atc cgg gct agt gat ttt gac tta att gct atg gtc aag Tyr Phe Ser Ile Arg Ala Ser Asp Phe Asp Leu Ile Ala Met Val Lys	3993

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aga gaa aaa act gcc aca gct aag gac aaa gcc ttg ctc acc gtc ctt Arg Glu Lys Thr Ala Thr Ala Lys Asp Lys Ala Leu Leu Thr Val Leu 1330 1335 1340			4089
gaa gta gcc aat gaa atg gtt gaa cgg ggt ttt gac ttc aag atg gtg Glu Val Ala Asn Glu Met Val Glu Arg Gly Phe Asp Phe Lys Met Val 1345 1350 1355 1360			4137
gac atc aac aag tcc caa gcc aaa gac ttt gtc atc gaa gac aat ggc Asp Ile Asn Lys Ser Gln Ala Lys Asp Phe Val Ile Glu Asp Asn Gly 1365 1370 1375			4185
ctt cgt gct cca ttt agg gca gtc cct tcc ttg ggg tcc agt gcc gcc Leu Arg Ala Pro Phe Arg Ala Val Pro Ser Leu Gly Ser Ser Ala Ala 1380 1385 1390			4233
cag gct gtc att gat gcc agg gag gac agc gac ttc ttg tcc aag gaa Gln Ala Val Ile Asp Ala Arg Glu Asp Ser Asp Phe Leu Ser Lys Glu 1395 1400 1405			4281
gac cta tca aaa cgg ggc aag ttg tcg aaa acg gtc atg gac tac ctg Asp Leu Ser Lys Arg Gly Lys Leu Ser Lys Thr Val Met Asp Tyr Leu 1410 1415 1420			4329
gac aat aac cac gtt tta gac cac ctg ccg gac gaa aac caa ctt tcc Asp Asn Asn His Val Leu Asp His Leu Pro Asp Glu Asn Gln Leu Ser 1425 1430 1435 1440			4377
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<211> 1444

<212> PRT

<213> Alloiococcus otitidis

<400> 70

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20 25 30

Leu Lys Gln Ile Val Val Tyr Lys Ala Gln Gln Ala Trp Asp Leu Thr
35 40 45

Leu Gln Phe Pro Gln Ile Leu Pro Phe Lys Asp Phe Gln Val Leu Glu

50

55

60

Ser Ala Leu Leu Gln His Ile Pro Glu Val Asn Gln Ile His Leu Arg
65 70 75 80

Val Asp Ala Gln Asp Asp Ser Phe Asp Gln Asp Leu Leu Gln Asp Tyr
85 90 95

Trp Pro Lys Ala Val Lys Phe Ser Gly Val Asp Ser Pro Leu Cys Asn
100 105 110

Asp Leu Leu Asp Lys Thr Leu Pro Tyr Leu Asp Gly Lys Gln Val Tyr
115 120 125

Phe Asp Leu Asp His Glu Val Thr Arg Asp Lys Phe Asp His Asp Phe
130 135 140

Leu Pro Arg Ile Gln Ala Gly Tyr Gln Gln Val Gly Phe Pro Asn His
145 150 155 160

Phe Lys Ile Lys Ala Arg Val Asp Ala Gln Lys Asn Ser Asp Gln Ile
165 170 175

Ala Ala Phe Arg Lys Glu Lys Glu Glu Lys Asp Gln Ala Leu Ser Gln
180 185 190

Glu Leu Thr Asn Gln Phe Ile Lys Ala Ser Gln Lys Lys Glu Glu Gly
195 200 205

Gly Ser Lys Ala Lys Ser Glu Ala Leu Lys Met Gly Arg Ala Ile Pro
210 215 220

Asp His Glu Thr Ile Thr Gln Met Val Asp Val Glu Glu Glu Glu Ser
225 230 235 240

Arg Leu Thr Phe Glu Gly Tyr Val Phe Asp Val Glu Ile Lys Ser Leu
245 250 255

Arg Ser Asp Arg Lys Leu Leu Leu Phe Lys Met Thr Asp Tyr Ser Ser
260 265 270

Ser Phe Leu Phe Lys Lys Phe Ser Asn Asn Ser Ser Asp Glu Ala Leu
275 280 285

Phe Asp Gln Val Gln Glu Gly Met Trp Leu Lys Val Arg Gly Ser Val
290 295 300

Gln Glu Asp Thr Phe Val Lys Asp Leu Val Val Met Ala Gln Asp Ile
305 310 315 320

Gln Glu Val Lys Lys Glu Pro Arg Arg Asp Leu Ala Lys Glu Gly Glu
325 330 335

Lys Arg Val Glu Leu His Ala His Thr Thr Met Ser Gln Met Asp Gly
340 345 350

Leu Val Pro Ala Lys Asp Leu Val Lys Gln Ala Ala Ala Phe Asp Gln
355 360 365

Pro Ala Ile Ala Ile Thr Asp His Ala Val Val Gln Ser Phe Pro Glu
370 375 380

Ala His Tyr Ala Gly Leu Asp Thr Gly Val Lys Ile Leu Tyr Gly Val
385 390 395 400

Glu Ala Asn Leu Val Ser Asp Gly Glu Leu Val Ala Tyr Asn Pro Ala
405 410 415

Asp Ile Lys Leu Glu Glu Ala Thr Tyr Val Val Phe Asp Val Glu Thr
420 425 430

Thr Gly Leu Ser Ala Arg Tyr Asp Gln Ile Ile Glu Leu Ala Ala Val
435 440 445

Lys Met Glu Asn Gly Glu Ile Val Ser Glu Phe Gln Glu Phe Ile Asp
450 455 460

Pro Gly Gln Pro Leu Ser Glu Thr Thr Thr Asn Leu Thr Gly Ile Thr
465 470 475 480

Asp Asp Met Val Gln Gly Ser Lys Ser Glu Asp Glu Val Leu His Ala
485 490 495

Phe Gln Ala Phe Ser Glu Gly Thr Val Leu Val Ala His Asn Ala Ser
500 505 510

Phe Asp Met Gly Phe Ile Asn Thr Ala Tyr Gln Arg His Gly Leu Gly
515 520 525

Gln Ala Asp Gln Pro Val Ile Asp Thr Leu Glu Leu Ser Arg Met Leu
530 535 540

His Pro Asn Leu Lys Ser His Arg Leu Asn Thr Leu Ala Lys Arg Tyr
545 550 555 560

Asp Val Ala Leu Glu His His His Arg Ala Ile Tyr Asp Ser Glu Ser
565 570 575

Thr Ala Lys Leu Leu Trp Ile Phe Leu Lys Glu Ala Lys Asp Gln Tyr
580 585 590

Asp Met Thr Ser His Gln Asp Leu Asn Ser Gln Val Gly Glu Glu Glu
595 600 605

Ala Tyr Lys Gln Ala Arg Pro Thr His Ala Ser Ile Leu Val Lys Asn
610 615 620

Gln Lys Gly Leu Lys Asn Leu Phe Lys Ile Val Ser His Ala His Val
625 630 635 640

Asn Tyr Phe Tyr Arg Val Pro Arg Ile Pro Lys Ser Ile Leu Ser Lys
645 650 655

Tyr Arg Glu Gly Leu Leu Val Gly Ser Gly Cys Gly Gln Gly Glu Leu
660 665 670

Phe Glu Ala Ile Met Gln Lys Gly Tyr Asp Glu Ala Leu Ala Val Ala
675 680 685

Gln Asp Tyr Asp Tyr Ile Glu Val Met Pro Lys Ser Ala Tyr Ile Asp
690 695 700

Leu Leu Asp Arg Asp Leu Ile Lys Asp Glu Ala Thr Leu Glu Glu Met
705 710 715 720

Ile Glu Asn Leu Val Lys Ile Gly His Glu Leu Asp Ile Pro Val Val
725 730 735

Ala Thr Gly Asn Val His Tyr Leu Asn Pro Glu Asp Ala Val Leu Arg
740 745 750

Asp Ile Leu Leu Glu Thr Ala Lys Lys Gly Ala Phe Ser Lys Ala Arg
755 760 765

Asn Pro Glu Val His Phe Arg Thr Thr Asp Glu Met Leu Glu Glu Phe
770 775 780

Ser Phe Leu Gly Gln Asp Gln Ala Tyr Glu Ile Val Val Thr Asn Thr
785 790 795 800

Gln Lys Ile Ala Asp Ser Ile Glu Ser Ile Ser Pro Val Lys Glu Gly
805 810 815

Leu Tyr Ala Pro Lys Met Glu Gly Ser Asp Gln Glu Ile Arg Gln Met
820 825 830

Ser Tyr Lys Gln Ala Lys Ala Leu Tyr Gly Asp Pro Leu Pro Ser Ile
835 840 845

Val Glu Glu Arg Leu Glu Lys Glu Leu Lys Ser Ile Ile Asp Asn Asn
850 855 860

Phe Ser Val Ile Tyr Leu Ile Ser Gln Lys Leu Val Lys Lys Ser Val
865 870 875 880

Glu Asp Gly Tyr Leu Val Gly Ser Arg Gly Ser Val Gly Ser Ser Phe
885 890 895

Val Ala Thr Met Thr Gly Ile Thr Glu Val Asn Pro Leu Pro Pro His
900 905 910

Tyr Arg Cys Pro Asn Cys Gln His Thr Glu Phe Phe Thr Asn Gly Glu
915 920 925

Val Gly Ser Gly Phe Asp Leu Glu Ala Lys Lys Cys Pro Glu Cys Gln
930 935 940

Ser Leu Met Glu Ser Asp Gly His Asp Ile Pro Phe Glu Thr Phe Leu
945 950 955 960

Gly Phe Asn Gly Asp Lys Val Pro Asp Ile Asp Leu Asn Phe Ser Gly

965

970

975

Glu Tyr Gln Ala Lys Ala His Asn Tyr Thr Lys Val Leu Phe Gly Glu
980 985 990

Asp His Val Tyr Arg Ala Gly Thr Ile Thr Thr Ile Ala Asp Lys Thr
995 1000 1005

Ala Phe Gly Phe Val Lys Gly Tyr Glu Arg Asp Lys Gln Ile Asn Tyr
1010 1015 1020

Arg Ser Ala Glu Val Asp Arg Leu Ser Asp Gly Leu Thr Gly Val Arg
1025 1030 1035 1040

Arg Ser Thr Gly Gln His Pro Gly Gly Ile Ile Val Ile Pro Asp Asp
1045 1050 1055

Met Asp Val Phe Asp Phe Thr Pro Ile Gln Tyr Pro Ala Asp Asp Gln
1060 1065 1070

Thr Ala Glu Trp Gln Thr Thr His Phe Asp Phe His Ser Ile Asp Glu
1075 1080 1085

Asn Val Leu Lys Leu Asp Ile Leu Gly His Asp Asp Pro Thr Met Ile
1090 1095 1100

Arg Lys Leu Gln Asp Leu Ser Gly Phe Asp Pro Gln Glu Ile Pro Val
1105 1110 1115 1120

Ser Asp Glu Asp Val Met Lys Ile Phe Ser Gly Pro Glu Val Leu Gly
1125 1130 1135

Val Thr Pro Glu Gln Ile Phe Ser Asn Thr Gly Thr Leu Gly Val Pro
1140 1145 1150

Glu Phe Gly Thr Gln Phe Val Arg Glu Met Leu Glu Gln Thr His Pro
1155 1160 1165

Ser Thr Phe Ala Glu Leu Leu Gln Ile Ser Gly Leu Ser His Gly Thr
1170 1175 1180

Asp Val Trp Leu Gly Asn Ala Glu Glu Leu Ile Arg Asn His Asn Ile
1185 1190 1195 1200

Pro Leu Ser Glu Val Ile Gly Cys Arg Asp Asp Ile Met Val Tyr Leu
1205 1210 1215

Gln His Gln Gly Leu Glu Asp Ser Leu Ala Phe Lys Ile Met Glu Phe
1220 1225 1230

Val Arg Lys Gly Arg Gly Leu Gln Asp Asp Trp Ile Ala Thr Met Lys
1235 1240 1245

Glu Asn Asp Val Pro Asp Trp Tyr Ile Glu Ser Cys Lys Lys Ile Lys
1250 1255 1260

Tyr Met Phe Pro Lys Ala His Ala Ala Ala Tyr Val Leu Met Ala Leu
1265 1270 1275 1280

Arg Val Ala Tyr Phe Lys Val His Tyr Pro Leu Tyr Tyr Tyr Ala Ala
1285 1290 1295

Tyr Phe Ser Ile Arg Ala Ser Asp Phe Asp Leu Ile Ala Met Val Lys
1300 1305 1310

Gly Lys Glu Gly Ile Lys Gly Ala Met Lys Glu Ile Arg Asp Lys Glu
1315 1320 1325

Arg Glu Lys Thr Ala Thr Ala Lys Asp Lys Ala Leu Leu Thr Val Leu
1330 1335 1340

Glu Val Ala Asn Glu Met Val Glu Arg Gly Phe Asp Phe Lys Met Val
1345 1350 1355 1360

Asp Ile Asn Lys Ser Gln Ala Lys Asp Phe Val Ile Glu Asp Asn Gly
1365 1370 1375

Leu Arg Ala Pro Phe Arg Ala Val Pro Ser Leu Gly Ser Ser Ala Ala
1380 1385 1390

Gln Ala Val Ile Asp Ala Arg Glu Asp Ser Asp Phe Leu Ser Lys Glu
1395 1400 1405

Asp Leu Ser Lys Arg Gly Lys Leu Ser Lys Thr Val Met Asp Tyr Leu
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Leu Arg Ala Gly Lys Gly Gly Asp Gly Met Val Ala Phe Arg Arg Glu	
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aag tat gag ccc aat ggt gga cca gca ggc ggc gac ggt ggc agt ggc	147
Lys Tyr Glu Pro Asn Gly Gly Pro Ala Gly Gly Asp Gly Gly Ser Gly	
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ggt aac att atc ttc aag gta gat gaa ggc ctc cgt acc ctg gta gac	195
Gly Asn Ile Ile Phe Lys Val Asp Glu Gly Leu Arg Thr Leu Val Asp	
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ttc cgc tac aac ccc cat ttt aag gca gat agt ggc caa aat ggt atg	243
Phe Arg Tyr Asn Pro His Phe Lys Ala Asp Ser Gly Gln Asn Gly Met	
60 65 70 75	
ccc aag ggg atg aat ggt aag aag gca gag gac ttg att atc agt gtc	291
Pro Lys Gly Met Asn Gly Lys Lys Ala Glu Asp Leu Ile Ile Ser Val	
80 85 90	
ccg cct gga acc att atc cgg gat gcc caa agt aag gct ata ctt gct	339
Pro Pro Gly Thr Ile Ile Arg Asp Ala Gln Ser Lys Ala Ile Leu Ala	
95 100 105	
gac tta caa gaa gaa gga caa gaa gtc ttg gca gcc caa ggt ggc cgg	387
Asp Leu Gln Glu Glu Gly Gln Glu Val Leu Ala Ala Gln Gly Gly Arg	
110 115 120	
gga ggt cgg ggc aat aaa cgt ttt gct acg cat aag aac cca gca ccc	435
Gly Gly Arg Gly Asn Lys Arg Phe Ala Thr His Lys Asn Pro Ala Pro	
125 130 135	
tcc att gcc gaa aac ggc gag ccg ggc caa gag cgg gat gtc gaa ttg	483
Ser Ile Ala Glu Asn Gly Glu Pro Gly Gln Glu Arg Asp Val Glu Leu	

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gaa tta aaa gtc atg gcc gat gtt ggc cta gtg ggt tat cct tct gtc Glu Leu Lys Val Met Ala Asp Val Gly Leu Val Gly Tyr Pro Ser Val 160 165 170				531
ggg aaa tcg acc ctt ttg tcg gtt gtc tca ggc gct aaa ccc aaa att Gly Lys Ser Thr Leu Leu Ser Val Val Ser Gly Ala Lys Pro Lys Ile 175 180 185				579
gga gcc tat cac ttt act aca ctt gcc cct aat tta ggt gta gtg aat Gly Ala Tyr His Phe Thr Thr Leu Ala Pro Asn Leu Gly Val Val Asn 190 195 200				627
gca gtg gac ggc aag gaa ttt gtc ttg gcg gat att cct ggc tta att Ala Val Asp Gly Lys Glu Phe Val Leu Ala Asp Ile Pro Gly Leu Ile 205 210 215				675
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att gaa aga acc cgc atc ctc ctt cat gta ctt gat atg agc gga atg Ile Glu Arg Thr Arg Ile Leu Leu His Val Leu Asp Met Ser Gly Met 240 245 250				771
gaa ggt cgc cat cca att gat gat ttt gac cag att aac caa gaa cta Glu Gly Arg His Pro Ile Asp Asp Phe Asp Gln Ile Asn Gln Glu Leu 255 260 265				819
aaa gac tat aat gag aaa tta ttg gac cgc aag cag gtc att gtg gcc Lys Asp Tyr Asn Glu Lys Leu Leu Asp Arg Lys Gln Val Ile Val Ala 270 275 280				867
aat aaa atg gac ctg ccc cag tcc cgg gat aat tta atc gaa ttt aaa Asn Lys Met Asp Leu Pro Gln Ser Arg Asp Asn Leu Ile Glu Phe Lys 285 290 295				915
gcc gag tta gac agc cgg gac ctt gac tat gaa atc ttt gaa gtg tca Ala Glu Leu Asp Ser Arg Asp Leu Asp Tyr Glu Ile Phe Glu Val Ser 300 305 310 315				963
gct gcc acc cag gct ggc att cag gac cta gtc atc cga cta gcc gac Ala Ala Thr Gln Ala Gly Ile Gln Asp Leu Val Ile Arg Leu Ala Asp 320 325 330				1011
tta gtc gac caa ctg gac caa gcc cca agt tta gac cag gaa gaa act Leu Val Asp Gln Leu Asp Gln Ala Pro Ser Leu Asp Gln Glu Glu Thr 335 340 345				1059
agt gaa gcc gac caa aga gtg gtc tac aag ttt caa gct gac caa gac Ser Glu Ala Asp Gln Arg Val Val Tyr Lys Phe Gln Ala Asp Gln Asp 350 355 360				1107
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 Pro Lys Val Glu Arg Leu Tyr Ala Met Thr Asn Phe Asp His Glu Glu
 380 385 390 395

gcc att atg cgg ttt tct cgc cag cta aga ggg atg gga gta gac caa 1251
 Ala Ile Met Arg Phe Ser Arg Gln Leu Arg Gly Met Gly Val Asp Gln
 400 405 410

gcc tta aga gac aag ggg gct cag tct ggt gac ctc gtc caa gtt gaa 1299
 Ala Leu Arg Asp Lys Gly Ala Gln Ser Gly Asp Leu Val Gln Val Glu
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<210> 72

<211> 435

<212> PRT

<213> *Alloiococcus otitidis*

<400> 72

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Gly Gly Pro Ala Gly Gly Asp Gly Gly Ser Gly Gly Asn Ile Ile Phe
 35 40 45

Lys Val Asp Glu Gly Leu Arg Thr Leu Val Asp Phe Arg Tyr Asn Pro
 50 55 60

His Phe Lys Ala Asp Ser Gly Gln Asn Gly Met Pro Lys Gly Met Asn
 65 70 75 80

Gly Lys Lys Ala Glu Asp Leu Ile Ile Ser Val Pro Pro Gly Thr Ile
 85 90 95

Ile Arg Asp Ala Gln Ser Lys Ala Ile Leu Ala Asp Leu Gln Glu Glu
 100 105 110

Gly Gln Glu Val Leu Ala Ala Gln Gly Gly Arg Gly Gly Arg Gly Asn
 115 120 125

Lys Arg Phe Ala Thr His Lys Asn Pro Ala Pro Ser Ile Ala Glu Asn

130

135

140

Gly Glu Pro Gly Gln Glu Arg Asp Val Glu Leu Glu Leu Lys Val Met
145 150 155 160

Ala Asp Val Gly Leu Val Gly Tyr Pro Ser Val Gly Lys Ser Thr Leu
165 170 175

Leu Ser Val Val Ser Gly Ala Lys Pro Lys Ile Gly Ala Tyr His Phe
180 185 190

Thr Thr Leu Ala Pro Asn Leu Gly Val Val Asn Ala Val Asp Gly Lys
195 200 205

Glu Phe Val Leu Ala Asp Ile Pro Gly Leu Ile Glu Gly Ala Ser Glu
210 215 220

Gly Val Gly Leu Gly Ile Asp Phe Leu Lys His Ile Glu Arg Thr Arg
225 230 235 240

Ile Leu Leu His Val Leu Asp Met Ser Gly Met Glu Gly Arg His Pro
245 250 255

Ile Asp Asp Phe Asp Gln Ile Asn Gln Glu Leu Lys Asp Tyr Asn Glu
260 265 270

Lys Leu Leu Asp Arg Lys Gln Val Ile Val Ala Asn Lys Met Asp Leu
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Pro Gln Ser Arg Asp Asn Leu Ile Glu Phe Lys Ala Glu Leu Asp Ser
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Arg Asp Leu Asp Tyr Glu Ile Phe Glu Val Ser Ala Ala Thr Gln Ala
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Gly Ile Gln Asp Leu Val Ile Arg Leu Ala Asp Leu Val Asp Gln Leu
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Asp Gln Ala Pro Ser Leu Asp Gln Glu Glu Thr Ser Glu Ala Asp Gln
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Arg Asp Pro Glu Gly Val Trp Leu Val Ser Gly Pro Lys Val Glu Arg
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Leu Tyr Ala Met Thr Asn Phe Asp His Glu Glu Ala Ile Met Arg Phe
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Ser Arg Gln Leu Arg Gly Met Gly Val Asp Gln Ala Leu Arg Asp Lys
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 Gly Arg Pro Asn Val Gly Lys Ser Thr Ile Phe Asn Arg Ile Ile Gly
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 Asp Arg Leu Ala Ile Val Gln Asp Glu Pro Gly Val Thr Arg Asp Arg
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 Ile Tyr Ala Asp Ala Glu Trp Leu Gly Lys Asp Phe Ser Val Ile Asp
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acg gga gga atc act ttt gat gat ttg ccc ttg cat gaa gaa ata aaa 243
 Thr Gly Gly Ile Thr Phe Asp Asp Leu Pro Leu His Glu Glu Ile Lys
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 Val Gln Ala Glu Ile Ala Ile Asp Glu Ala Asp Val Ile Val Met Val
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Thr Ser Val Lys Glu Gly Ile Thr Asp Leu Asp Asp Gln Val Ala Leu	
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Ile Leu Gln Gln Ser Asn Lys Pro Val Val Leu Ala Val Asn Lys Thr	
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Asp Asn Pro Glu Leu Arg Asn Glu Ile Tyr Glu Phe Tyr Gly Leu Gly	
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Ala Tyr Asp Gln Asp Thr Ile Lys Phe Cys Leu Ile Gly Arg Pro Asn	
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Val Gly Lys Ser Ser Leu Val Asn Ala Ile Ile Gly Glu Asp Arg Val	
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Ile Val Ser Glu Leu Glu Gly Thr Thr Arg Asp Ala Ile Asp Thr Pro	
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Phe Met Thr Gln Asp Gly Gln Asp Tyr Val Met Ile Asp Thr Ala Gly	
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Leu Asp Ala Glu Thr Gly Ile Arg Asp Gln Asp Lys Lys Val Phe Gly	
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Tyr Ala His Gln Ala Gly Lys Gly Ile Ile Ile Leu Val Asn Lys Trp	
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Asp Thr Ile Lys Lys Glu Thr Asn Thr Met Arg Asp Phe Glu Leu Gln	
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Ile Arg Asp Gln Phe Arg Tyr Leu His Tyr Ala Pro Ile Leu Phe Val
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 Ser Ala Lys Thr Lys Gln Arg Leu Glu Val Ile Pro Glu Leu Val Asp
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 Asp Val Leu Ser Asp Ala Leu Ala Ser Asn Pro Ala Pro Ser Lys Ser
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 Gly Lys Arg Leu Lys Val Phe Tyr Ala Thr Gln Val Ala Thr Asn Pro
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cct act ttt gtg gtt ttt gtc aat gat cct gac ctc atg cac ttc tcc 1251
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tat gag cgc ttt tta gaa aat cga ttc cgc gaa agc ttt gac ttc tat 1299
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<213> Alloiococcus otitidis

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Leu Gly Lys Asp Phe Ser Val Ile Asp Thr Gly Gly Ile Thr Phe Asp
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Asp Leu Pro Leu His Glu Glu Ile Lys Val Gln Ala Glu Ile Ala Ile
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Asp Glu Ala Asp Val Ile Val Met Val Thr Ser Val Lys Glu Gly Ile
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Thr Asp Leu Asp Asp Gln Val Ala Leu Ile Leu Gln Gln Ser Asn Lys
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Pro Val Val Leu Ala Val Asn Lys Thr Asp Asn Pro Glu Leu Arg Asn
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Glu Ile Tyr Glu Phe Tyr Gly Leu Gly Leu Gly Asp Pro Leu Pro Val
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Ser Gly Ser His Gly Leu Gly Phe Gly Asp Leu Leu Asp Ala Val Val
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Ala Asn Phe Pro Asn Glu Ala Asn Met Ala Tyr Asp Gln Asp Thr Ile
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Lys Phe Cys Leu Ile Gly Arg Pro Asn Val Gly Lys Ser Ser Leu Val
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Asn Ala Ile Ile Gly Glu Asp Arg Val Ile Val Ser Glu Leu Glu Gly
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Thr Thr Arg Asp Ala Ile Asp Thr Pro Phe Met Thr Gln Asp Gly Gln
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Asp Tyr Val Met Ile Asp Thr Ala Gly Ile Arg Arg Arg Gly Lys Val
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Tyr Glu Lys Thr Glu Lys Tyr Ser Val Met Arg Ala Gln Arg Ala Ile
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260 265 270

Arg Asp Gln Asp Lys Lys Val Phe Gly Tyr Ala His Gln Ala Gly Lys
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Asn Thr Met Arg Asp Phe Glu Leu Gln Ile Arg Asp Gln Phe Arg Tyr
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Leu His Tyr Ala Pro Ile Leu Phe Val Ser Ala Lys Thr Lys Gln Arg
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Leu Glu Val Ile Pro Glu Leu Val Asp Arg Val Tyr Tyr Asn Arg Asn
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Gln Arg Val Lys Ser Ser Leu Leu Asn Asp Val Leu Ser Asp Ala Leu
355 360 365

Ala Ser Asn Pro Ala Pro Ser Lys Ser Gly Lys Arg Leu Lys Val Phe
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Tyr Ala Thr Gln Val Ala Thr Asn Pro Pro Thr Phe Val Val Phe Val
385 390 395 400

Asn Asp Pro Asp Leu Met His Phe Ser Tyr Glu Arg Phe Leu Glu Asn
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Phe Thr His Leu Gln Val Thr Ser Ala Tyr Thr Leu Met Ala Ser Thr
15 20 25 30

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Ile Gln Leu Pro Leu Leu Met Asp Arg Leu Lys Glu Leu Gly Met Glu

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Tyr	Gln	Glu	Ala	Lys	Lys	His	Gly	Ile	Lys	Pro	Ile	Met	Gly	Leu	Arg					
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Ala	Gly	Tyr	Gln	Ala	Leu	Leu	Ala	Leu	Ser	Thr	Asp	Leu	Gln	Val	Asn					
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Lys	Gln	Ala	Ile	Thr	Leu	Asp	Gln	Val	Arg	Ser	Val	Ala	Gln	Asp	Leu					
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Tyr	Thr	Ile	Phe	Pro	Ser	Ser	Asp	Pro	Lys	Val	Lys	Ala	Asp	Leu	Leu					
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Ala	Arg	Thr	Leu	Ser	Asp	Ser	Gly	Gly	Leu	Lys	Val	Leu	Ala	Leu	Ser					
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Gln	Val	Gly	Leu	Gly	Gln	Ala	Leu	Lys	Asn	Thr	Lys	Asp	Val	Ala	Gln					
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Val Val Met Ser Asp Gln Pro Leu Ile His Ser Leu Pro Leu Gln Asp	
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Gly Asn Gly Lys Val Pro Asn Thr Gln Phe Thr Met Glu Asp Val Glu	
515 520 525	
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Ala Val Gly Leu Leu Lys Met Asp Phe Leu Ser Leu Lys Asn Leu Thr	
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Ile Leu Ala Asp Cys Leu Asn Phe Ser Gln Tyr Glu Gly Gln Gly Gly	
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Leu Phe Ala Arg Gly Asp Thr Asn Gly Val Phe Gln Phe Glu Lys Glu	
575 580 585 590	
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655 660 665 670	
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Lys Gly Tyr Ser Glu Ser Val Ala Arg Glu Val Tyr Asn Tyr Ile Ala	
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Asn	His	Ile	Leu	Glu	Ile	Arg	Lys	Glu	Lys	Gly	Ala	Phe	Thr	Ser	Leu	
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Arg	Asp	Phe	Cys	Glu	Lys	Ile	Asp	Ser	Gln	Phe	Leu	Ser	Gln	Asp	Pro	
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Ile	Glu	Ala	Leu	Ile	Leu	Val	Gly	Ala	Phe	Asp	Gln	Met	Gly	Pro	Asn	
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cgg	cgg	acc	atg	tta	gcg	ggc	ttg	gaa	gca	acg	att	gaa	ttc	gtg	gcc	2643
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Ser	Leu	Arg	Gln	Asp	Leu	Lys	Thr	Ser	Phe	Ile	Ala	Asp	Leu	Glu	Glu	
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cta cct tac ctc aaa gaa gga gtg gtc ctg gtc gtc tca ggc aag gta Leu Pro Tyr Leu Lys Glu Gly Val Val Leu Val Val Ser Gly Lys Val 995 1000 1005			3027
gaa gtt agg aag gga gaa atc cag cta aaa gtc cag acc atg aaa gag Glu Val Arg Lys Gly Glu Ile Gln Leu Lys Val Gln Thr Met Lys Glu 1010 1015 1020			3075
gcc agc cag gtc caa aaa gag act aag cag ctt tac ctg aaa ttt gct Ala Ser Gln Val Gln Lys Glu Thr Lys Gln Leu Tyr Leu Lys Phe Ala 1025 1030 1035			3123
gac ttg aac caa gat aaa gaa agt ttt cgt caa gtg caa aag atc ttg Asp Leu Asn Gln Asp Lys Glu Ser Phe Arg Gln Val Gln Lys Ile Leu 1040 1045 1050			3171
gcc cga cat ccc ggc cag aag cga gtg att gtt tac gac cag gcc agc Ala Arg His Pro Gly Gln Lys Arg Val Ile Val Tyr Asp Gln Ala Ser 1055 1060 1065 1070			3219
cag caa gca ctc cag ctc aaa gca aaa ttt aat ttc gac gga cgg acg Gln Gln Ala Leu Gln Leu Lys Ala Lys Phe Asn Phe Asp Gly Arg Thr 1075 1080 1085			3267
gat acc cta aac cag ctc cag gac ctc cta ggc cag gat tct tgt atc Asp Thr Leu Asn Gln Leu Gln Asp Leu Leu Gly Gln Asp Ser Cys Ile 1090 1095 1100			3315
tta aaa taa Leu Lys:			3324

<210> 76

<211> 1104

<212> PRT

<213> Alloiococcus otitidis

<400> 76

Met	Lys	Gln	Ile	Cys	Leu	Arg	Arg	Arg	Gly	Asp	Lys	Met	Thr	Phe	Thr
1				5					10					15	

His	Leu	Gln	Val	Thr	Ser	Ala	Tyr	Thr	Leu	Met	Ala	Ser	Thr	Ile	Gln
			20					25					30		

Leu	Pro	Leu	Leu	Met	Asp	Arg	Leu	Lys	Glu	Leu	Gly	Met	Glu	Ala	Val
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

35

40

45

Ala Leu Thr Asp His Asn Val Met His Gly Ala Val Glu Phe Tyr Gln
50 55 60

Glu Ala Lys Lys His Gly Ile Lys Pro Ile Met Gly Leu Arg Ala Asp
65 70 75 80

Leu Asp Glu Gly Ile Thr Val Thr Leu Leu Ala Lys Asn Lys Ala Gly
85 90 95

Tyr Gln Ala Leu Leu Ala Leu Ser Thr Asp Leu Gln Val Asn Lys Gln
100 105 110

Ala Ile Thr Leu Asp Gln Val Arg Ser Val Ala Gln Asp Leu Tyr Thr
115 120 125

Ile Phe Pro Ser Ser Asp Pro Lys Val Lys Ala Asp Leu Leu Asp Lys
130 135 140

Gln Ala Ser Asn Leu Thr Ala Met Thr Gln Asn Leu Pro His Ser Tyr
145 150 155 160

Leu Gly Leu Val Pro Asp Gln Asp Gln Lys Ile Tyr Gln Leu Ala Arg
165 170 175

Thr Leu Ser Asp Ser Gly Gly Leu Lys Val Leu Ala Leu Ser Asp Val
180 185 190

Arg Cys Leu Glu Glu Ser Gln Val Ser Thr Leu Glu Ile Leu Ser His
195 200 205

Ile Lys Ala Asn Gln Lys Ile Gln Phe Asp Thr Gln Ala Arg Glu Asn
210 215 220

Tyr Ala Leu Arg Ser Pro Gln Glu Met Glu Ser Phe Phe Asn Gln Val
225 230 235 240

Gly Leu Gly Gln Ala Leu Lys Asn Thr Lys Asp Val Ala Gln Ser Val
245 250 255

Asp Trp Ser Leu Asp Leu Gly Gln Ala Lys Leu Pro Ala Phe Asp Leu
260 265 270

Pro Glu Gly Glu Thr Lys Asp Ser Tyr Leu Gly Lys Leu Ala Gln Lys
275 280 285

Gly Leu Gln Glu Arg Val Pro Gly Tyr Gly Gln Asp Tyr Gln Asp Arg
290 295 300

Leu Asp Lys Glu Leu Ala Val Ile Ser Ser Met Gly Phe Ser Asp Tyr
305 310 315 320

Phe Leu Ile Val Trp Asp Leu Met Gln Phe Ala Arg Gln Glu Lys Ile
325 330 335

Glu Thr Gly Phe Gly Arg Gly Ser Ala Ala Ala Ser Leu Val Ser Tyr
340 345 350

Ala Leu Tyr Ile Thr Gly Val Asp Pro Ile His Tyr Asp Leu Leu Phe
355 360 365

Glu Arg Phe Leu Asn Lys Asp Arg Phe Thr Met Pro Asp Ile Asp Leu
370 375 380

Asp Phe Pro Asp Asn Lys Arg Gln Val Ile Leu Asp Tyr Val Tyr Arg
385 390 395 400

Lys Tyr Gly Pro Asp His Val Ala Gln Ile Leu Thr Phe Gly Thr Phe
405 410 415

Ala Ala Lys Ser Ser Ile Arg Glu Ile Met Arg Thr Leu Gly Tyr Lys
420 425 430

Asn Glu Asp Met Lys Thr Trp Ser Gln Ala Ile Pro Asp Thr Val Asn
435 440 445

Ile Ser Leu Ser Lys Ala Tyr Asp Glu Ser Lys Asp Leu Gln Lys Leu
450 455 460

Val Gln Gln Ser His Glu Asn Glu Arg Ile Phe Ala Met Ala Gln Asp
465 470 475 480

Ile Glu Gly Leu Pro Arg Asn Tyr Ser Thr His Ala Ala Gly Val Val
485 490 495

Met Ser Asp Gln Pro Leu Ile His Ser Leu Pro Leu Gln Asp Gly Asn
500 505 510

Gly Lys Val Pro Asn Thr Gln Phe Thr Met Glu Asp Val Glu Ala Val
515 520 525

Gly Leu Leu Lys Met Asp Phe Leu Ser Leu Lys Asn Leu Thr Ile Leu
530 535 540

Ala Asp Cys Leu Asn Phe Ser Gln Tyr Glu Gly Gln Gly Gly Gly Ile
545 550 555 560

Ser Lys Gln Asp Ile Pro Ile Asp Asp Pro Lys Thr Leu Asp Leu Phe
565 570 575

Ala Arg Gly Asp Thr Asn Gly Val Phe Gln Phe Glu Lys Glu Gly Ile
580 585 590

Lys Lys Val Leu Arg Gln Leu Gln Pro Thr Ser Phe Glu Asp Ile Val
595 600 605

Ala Thr Asn Ala Leu Tyr Arg Pro Gly Pro Met Gly Gln Ile Glu Asn
610 615 620

Tyr Ile Asn Arg Lys His Gly Gln Glu Lys Ile Ile Tyr Pro His Glu
625 630 635 640

Asp Leu Lys Asp Ile Leu Glu Val Thr Tyr Gly Ile Ile Val Tyr Gln
645 650 655

Glu Gln Val Met Gln Val Ala Thr Gln Leu Ala Gly Tyr Ser Leu Ser
660 665 670

Glu Ala Asp Gln Leu Arg Arg Thr Met Ser Lys Lys Ile Gln Ser Glu
675 680 685

Met Asp Gln Gly Arg Glu Lys Phe Ile Arg Gly Ala Leu Asp Lys Gly
690 695 700

Tyr Ser Glu Ser Val Ala Arg Glu Val Tyr Asn Tyr Ile Ala Lys Phe
705 710 715 720

Ala Asn Tyr Gly Phe Asn Arg Ala His Ala Val Ala Tyr Ser Met Leu
725 730 735

Ala Tyr His Met Ala Tyr Phe Lys Val His Gln Pro Lys Ser Phe Phe
740 745 750

Ala Ala Val Met Lys Ala Asp Trp Gly Asn Lys Ala Lys Ile Tyr Lys
755 760 765

Tyr Ala His Glu Val Arg Ala Arg Lys Ile Lys Leu Leu Lys Pro Asp
770 775 780

Ile Asn Gln Ser Leu Gly Ser Phe Thr Val Arg Gln Asn Gly Ile Gln
785 790 795 800

Val Gly Leu Lys Met Val Lys Gly Val Ala Ser Pro Phe Val Asn His
805 810 815

Ile Leu Glu Ile Arg Lys Glu Lys Gly Ala Phe Thr Ser Leu Arg Asp
820 825 830

Phe Cys Glu Lys Ile Asp Ser Gln Phe Leu Ser Gln Asp Pro Ile Glu
835 840 845

Ala Leu Ile Leu Val Gly Ala Phe Asp Gln Met Gly Pro Asn Arg Arg
850 855 860

Thr Met Leu Ala Gly Leu Glu Ala Thr Ile Glu Phe Val Ala Lys Ser
865 870 875 880

Ser Gly Asn Ile Thr Leu Phe Asp Thr Leu Lys Pro Arg Gln Glu Asp
885 890 895

Leu Glu Glu Phe Ser Pro Lys Asp Leu Ile Gln Tyr Glu Glu Glu Leu
900 905 910

Thr Gly Phe Tyr Phe Ser Ser His Pro Leu Ser Arg Tyr Asp Ser Leu
915 920 925

Arg Gln Asp Leu Lys Thr Ser Phe Ile Ala Asp Leu Glu Glu Gly Gln
930 935 940

Ser Cys Gln Val Leu Gly Gln Leu Val Gln Val Arg Lys Thr Gln Thr

945	950	955	960
Arg Asn Gln Gln Pro Met Ala Phe Val Ser Leu Ala Asp Gln Thr Gly	965	970	975
Gln Ile Ser Leu Val Val Phe Pro Asn Val Tyr Arg Glu Cys Leu Pro	980	985	990
Tyr Leu Lys Glu Gly Val Val Leu Val Val Ser Gly Lys Val Glu Val	995	1000	1005
Arg Lys Gly Glu Ile Gln Leu Lys Val Gln Thr Met Lys Glu Ala Ser	1010	1015	1020
Gln Val Gln Lys Glu Thr Lys Gln Leu Tyr Leu Lys Phe Ala Asp Leu	1025	1030	1035
Asn Gln Asp Lys Glu Ser Phe Arg Gln Val Gln Lys Ile Leu Ala Arg	1045	1050	1055
His Pro Gly Gln Lys Arg Val Ile Val Tyr Asp Gln Ala Ser Gln Gln	1060	1065	1070
Ala Leu Gln Leu Lys Ala Lys Phe Asn Phe Asp Gly Arg Thr Asp Thr	1075	1080	1085
Leu Asn Gln Leu Gln Asp Leu Leu Gly Gln Asp Ser Cys Ile Leu Lys	1090	1095	1100

<210> 77
 <211> 1212
 <212> DNA
 <213> *Alloiococcus otitidis*

<220>
 <221> CDS
 <222> (7)..(1212)
 <223>

<400> 77
 acaaag atg ctg aaa aat aaa aag ata gcc tta tat gtt act ggt ggt
 Met Leu Lys Asn Lys Lys Ile Ala Leu Tyr Val Thr Gly Gly
 1 5 10

ata gca gta tac aaa tca ctt tac tta ctt agg gaa atc atc aaa caa Ile Ala Val Tyr Lys Ser Leu Tyr Leu Leu Arg Glu Ile Ile Lys Gln 15 20 25 30	96
ggc ggg gag gtc cgg gtt gcc atg act caa gca gct tgt caa ttt gtt Gly Gly Glu Val Arg Val Ala Met Thr Gln Ala Ala Cys Gln Phe Val 35 40 45	144
aac ccc tta tct ttt cag gtt tta agc caa aaa aag gtt cag att gac Asn Pro Leu Ser Phe Gln Val Leu Ser Gln Lys Lys Val Gln Ile Asp 50 55 60	192
act ttt gaa gaa ggt cag ccc gaa tcg gtc agt cac att gat ttg acg Thr Phe Glu Glu Gly Gln Pro Glu Ser Val Ser His Ile Asp Leu Thr 65 70 75	240
gat tgg gcc gac tac tcc atc gtg gct ccg gca act gcc aat atc atc Asp Trp Ala Asp Tyr Ser Ile Val Ala Pro Ala Thr Ala Asn Ile Ile 80 85 90	288
ggc aag ctg gcc aat ggg att ggg gac gat ttt gtt tca aca gcc ttg Gly Lys Leu Ala Asn Gly Ile Gly Asp Asp Phe Val Ser Thr Ala Leu 95 100 105 110	336
ttg gca acg gac cac ccc att ttt tta gtc cca gcc atg aac acc aag Leu Ala Thr Asp His Pro Ile Phe Leu Val Pro Ala Met Asn Thr Lys 115 120 125	384
atg tat gaa aat ccc gct ctt aag aaa aac aag gcc ttc ctt att gaa Met Tyr Glu Asn Pro Ala Leu Lys Lys Asn Lys Ala Phe Leu Ile Glu 130 135 140	432
cag ggc cat tac tgg atg gag ccg gat att gga ttt tta gca gag ggc Gln Gly His Tyr Trp Met Glu Pro Asp Ile Gly Phe Leu Ala Glu Gly 145 150 155	480
tac gaa ggc ttg ggt cgt ttt cca gac cta gac cgg att atg gcg gaa Tyr Glu Gly Leu Gly Arg Phe Pro Asp Leu Asp Arg Ile Met Ala Glu 160 165 170	528
ttt aac cat ttt att att gct agg aat cca ggt atc cta tca gga aaa Phe Asn His Phe Ile Ile Ala Arg Asn Pro Gly Ile Leu Ser Gly Lys 175 180 185 190	576
aaa gtc ctc gtc aca gca ggt ggg acg gtg gag cgg att gat ccc gtc Lys Val Leu Val Thr Ala Gly Gly Thr Val Glu Arg Ile Asp Pro Val 195 200 205	624
cgg tat att tcc aat gat tct tct ggt aag atg ggc cac caa ctt gct Arg Tyr Ile Ser Asn Asp Ser Ser Gly Lys Met Gly His Gln Leu Ala 210 215 220	672
caa gcg gcc tat gaa gct ggg gcc cag gtt agc ttg gta aca gcc agt Gln Ala Ala Tyr Glu Ala Gly Ala Gln Val Ser Leu Val Thr Ala Ser 225 230 235	720
gac ttg ccg acc agt ccc ttt att gac cgc ttt cag gtg gag tcc acc	768

Asp	Leu	Pro	Thr	Ser	Pro	Phe	Ile	Asp	Arg	Phe	Gln	Val	Glu	Ser	Thr		
240						245					250						
tta	gac	ttg	tac	caa	aca	gtt	agt	gac	ctc	tat	gac	cac	cat	gac	att	816	
Leu	Asp	Leu	Tyr	Gln	Thr	Val	Ser	Asp	Leu	Tyr	Asp	His	His	Asp	Ile		
255					260					265					270		
ctc	atg	atg	gcc	gca	gcg	gtg	tct	gac	tac	cgg	cca	gtc	aac	cgg	tca	864	
Leu	Met	Met	Ala	Ala	Ala	Val	Ser	Asp	Tyr	Arg	Pro	Val	Asn	Arg	Ser		
				275					280					285			
gac	aaa	aag	atg	aaa	aag	caa	gat	aat	tta	acc	att	gaa	ctg	gaa	aaa	912	
Asp	Lys	Lys	Met	Lys	Lys	Gln	Asp	Asn	Leu	Thr	Ile	Glu	Leu	Glu	Lys		
			290					295					300				
aat	cct	gat	att	ttg	gcc	gaa	atg	ggc	cgg	cgg	aaa	gac	caa	caa	atc	960	
Asn	Pro	Asp	Ile	Leu	Ala	Glu	Met	Gly	Arg	Arg	Lys	Asp	Gln	Gln	Ile		
		305					310					315					
aat	gtc	ggc	ttt	gca	gca	gaa	acc	cat	aac	ctt	gaa	gaa	tat	gcc	caa	1008	
Asn	Val	Gly	Phe	Ala	Ala	Glu	Thr	His	Asn	Leu	Glu	Glu	Tyr	Ala	Gln		
	320					325					330						
aaa	aaa	tta	gcc	tcc	aaa	caa	gct	gac	ttg	atc	gta	gcc	aat	gaa	gtg	1056	
Lys	Lys	Leu	Ala	Ser	Lys	Gln	Ala	Asp	Leu	Ile	Val	Ala	Asn	Glu	Val		
335					340					345					350		
ggc	cgg	gga	gac	cgg	ggc	ttt	aat	gcg	gat	gaa	aat	gcg	gcc	ctt	gtt	1104	
Gly	Arg	Gly	Asp	Arg	Gly	Phe	Asn	Ala	Asp	Glu	Asn	Ala	Ala	Leu	Val		
				355					360					365			
ttt	tcc	agt	gac	caa	gat	ccg	ctt	gag	ctt	ccc	ctt	cag	tct	aaa	aaa	1152	
Phe	Ser	Ser	Asp	Gln	Asp	Pro	Leu	Glu	Leu	Pro	Leu	Gln	Ser	Lys	Lys		
			370					375					380				
gat	atg	gca	aaa	aag	att	att	gaa	gtg	gtg	gcc	agt	aaa	ttg	cct	gct	1200	
Asp	Met	Ala	Lys	Lys	Ile	Ile	Glu	Val	Val	Ala	Ser	Lys	Leu	Pro	Ala		
		385					390					395					
tct	ccc	aaa	taa													1212	
Ser	Pro	Lys															
		400															

<210> 78

<211> 401

<212> PRT

<213> Alloiococcus otitidis

<400> 78

Met	Leu	Lys	Asn	Lys	Lys	Ile	Ala	Leu	Tyr	Val	Thr	Gly	Gly	Ile	Ala
1				5					10					15	

Val	Tyr	Lys	Ser	Leu	Tyr	Leu	Leu	Arg	Glu	Ile	Ile	Lys	Gln	Gly	Gly
			20					25					30		

Glu Val Arg Val Ala Met Thr Gln Ala Ala Cys Gln Phe Val Asn Pro
35 40 45

Leu Ser Phe Gln Val Leu Ser Gln Lys Lys Val Gln Ile Asp Thr Phe
50 55 60

Glu Glu Gly Gln Pro Glu Ser Val Ser His Ile Asp Leu Thr Asp Trp
65 70 75 80

Ala Asp Tyr Ser Ile Val Ala Pro Ala Thr Ala Asn Ile Ile Gly Lys
85 90 95

Leu Ala Asn Gly Ile Gly Asp Asp Phe Val Ser Thr Ala Leu Leu Ala
100 105 110

Thr Asp His Pro Ile Phe Leu Val Pro Ala Met Asn Thr Lys Met Tyr
115 120 125

Glu Asn Pro Ala Leu Lys Lys Asn Lys Ala Phe Leu Ile Glu Gln Gly
130 135 140

His Tyr Trp Met Glu Pro Asp Ile Gly Phe Leu Ala Glu Gly Tyr Glu
145 150 155 160

Gly Leu Gly Arg Phe Pro Asp Leu Asp Arg Ile Met Ala Glu Phe Asn
165 170 175

His Phe Ile Ile Ala Arg Asn Pro Gly Ile Leu Ser Gly Lys Lys Val
180 185 190

Leu Val Thr Ala Gly Gly Thr Val Glu Arg Ile Asp Pro Val Arg Tyr
195 200 205

Ile Ser Asn Asp Ser Ser Gly Lys Met Gly His Gln Leu Ala Gln Ala
210 215 220

Ala Tyr Glu Ala Gly Ala Gln Val Ser Leu Val Thr Ala Ser Asp Leu
225 230 235 240

Pro Thr Ser Pro Phe Ile Asp Arg Phe Gln Val Glu Ser Thr Leu Asp
245 250 255

Leu Tyr Gln Thr Val Ser Asp Leu Tyr Asp His His Asp Ile Leu Met
 260 265 270

Met Ala Ala Ala Val Ser Asp Tyr Arg Pro Val Asn Arg Ser Asp Lys
 275 280 285

Lys Met Lys Lys Gln Asp Asn Leu Thr Ile Glu Leu Glu Lys Asn Pro
 290 295 300

Asp Ile Leu Ala Glu Met Gly Arg Arg Lys Asp Gln Gln Ile Asn Val
 305 310 315 320

Gly Phe Ala Ala Glu Thr His Asn Leu Glu Glu Tyr Ala Gln Lys Lys
 325 330 335

Leu Ala Ser Lys Gln Ala Asp Leu Ile Val Ala Asn Glu Val Gly Arg
 340 345 350

Gly Asp Arg Gly Phe Asn Ala Asp Glu Asn Ala Ala Leu Val Phe Ser
 355 360 365

Ser Asp Gln Asp Pro Leu Glu Leu Pro Leu Gln Ser Lys Lys Asp Met
 370 375 380

Ala Lys Lys Ile Ile Glu Val Val Ala Ser Lys Leu Pro Ala Ser Pro
 385 390 395 400

Lys

<210> 79
 <211> 1053
 <212> DNA
 <213> *Alloiococcus otitidis*

<220>
 <221> CDS
 <222> (22) .. (1053)
 <223>

<400> 79
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 Met Lys Ile Glu Asp Gln Leu Lys Lys Ile
 1 5 10

aaa gac caa gac ttg tct ccc ctc tac ctg gtc cag gga gat gac cag 99
 Lys Asp Gln Asp Leu Ser Pro Leu Tyr Leu Val Gln Gly Asp Asp Gln
 15 20 25

tac ttg tta gac cag gtt aaa aaa agt ttg agc cag gcc ctt ttg gac Tyr Leu Leu Asp Gln Val Lys Lys Ser Leu Ser Gln Ala Leu Leu Asp 30 35 40	147
cag gat gaa gct tct atg aat ttt ggt caa ttt aat atg atg gct gat Gln Asp Glu Ala Ser Met Asn Phe Gly Gln Phe Asn Met Met Ala Asp 45 50 55	195
agc cta gac atg gcc ttg tct gat gcg gaa tcc tat ccc ttt ttt ggg Ser Leu Asp Met Ala Leu Ser Asp Ala Glu Ser Tyr Pro Phe Phe Gly 60 65 70	243
gac aag cgc ctg gtt tac atc caa gac ccc ttt ttc cta aca ggg gag Asp Lys Arg Leu Val Tyr Ile Gln Asp Pro Phe Phe Leu Thr Gly Glu 75 80 85 90	291
aag cgg aaa aca gat ctg gac cat gac ttg gat cgc ttg ctg gct tac Lys Arg Lys Thr Asp Leu Asp His Asp Leu Asp Arg Leu Leu Ala Tyr 95 100 105	339
ctc caa aac cca gcc gac ttt act gtt ctc gtc ttc ttt gcc ccc tat Leu Gln Asn Pro Ala Asp Phe Thr Val Leu Val Phe Phe Ala Pro Tyr 110 115 120	387
gag aaa ctg gac aag cgg aag aag gtc acc aaa gcc cta ttg cag gaa Glu Lys Leu Asp Lys Arg Lys Lys Val Thr Lys Ala Leu Leu Gln Glu 125 130 135	435
gct gag att ata gat gcc agt tcc cca gac caa aga gat cta aaa gat Ala Glu Ile Ile Asp Ala Ser Ser Pro Asp Gln Arg Asp Leu Lys Asp 140 145 150	483
atg gtc cag aaa aaa gta aag gct cga ggc tac cag ttt gac aaa gga Met Val Gln Lys Lys Val Lys Ala Arg Gly Tyr Gln Phe Asp Lys Gly 155 160 165 170	531
gct tta aag gcc ctg gtt gaa aaa acc aat gcc aac tta agt cgg gtc Ala Leu Lys Ala Leu Val Glu Lys Thr Asn Ala Asn Leu Ser Arg Val 175 180 185	579
atg caa gag ttg gac aag tta ttc ttg tac cat tta gat gac aaa atc Met Gln Glu Leu Asp Lys Leu Phe Leu Tyr His Leu Asp Asp Lys Ile 190 195 200	627
atc acc gtc cag tca gtt gac cag gtc gta tca cca agc ctg gaa agt Ile Thr Val Gln Ser Val Asp Gln Val Val Ser Pro Ser Leu Glu Ser 205 210 215	675
aat gtc ttt agt att aac gac tat att tta agc ggg caa agc cag gct Asn Val Phe Ser Ile Asn Asp Tyr Ile Leu Ser Gly Gln Ser Gln Ala 220 225 230	723
gct ata cgg gcc ttt aat gac tta att caa caa aag gaa gag cca att Ala Ile Arg Ala Phe Asn Asp Leu Ile Gln Gln Lys Glu Glu Pro Ile 235 240 245 250	771

aaa atc atc gcc att atg atg aac caa ttc cgt tta tta ttg cag gtt 819
 Lys Ile Ile Ala Ile Met Met Asn Gln Phe Arg Leu Leu Leu Gln Val
 255 260 265
 aaa ata ttg cgg act aag ggc tac caa caa gga gag atc gct aaa atc 867
 Lys Ile Leu Arg Thr Lys Gly Tyr Gln Gln Gly Glu Ile Ala Lys Ile
 270 275 280
 tta aaa gtt cac ccc tac cgg gtt aag cta gcc ata gag aaa cag gag 915
 Leu Lys Val His Pro Tyr Arg Val Lys Leu Ala Ile Glu Lys Gln Glu
 285 290 295
 att ttt tcc aag caa agt cta tcg acc gcc tac cgc tac tta att gag 963
 Ile Phe Ser Lys Gln Ser Leu Ser Thr Ala Tyr Arg Tyr Leu Ile Glu
 300 305 310
 tca gat cat ttg att aaa acg ggc aag gtg acc tcg caa ttg caa ttt 1011
 Ser Asp His Leu Ile Lys Thr Gly Lys Val Thr Ser Gln Leu Gln Phe
 315 320 325 330
 gaa ctt ttt gcc cta caa ttt aaa gat tct gtc atg aat taa 1053
 Glu Leu Phe Ala Leu Gln Phe Lys Asp Ser Val Met Asn
 335 340

<210> 80

<211> 343

<212> PRT

<213> *Alloiococcus otitidis*

<400> 80

Met Lys Ile Glu Asp Gln Leu Lys Lys Ile Lys Asp Gln Asp Leu Ser
 1 5 10 15

Pro Leu Tyr Leu Val Gln Gly Asp Asp Gln Tyr Leu Leu Asp Gln Val
 20 25 30

Lys Lys Ser Leu Ser Gln Ala Leu Leu Asp Gln Asp Glu Ala Ser Met
 35 40 45

Asn Phe Gly Gln Phe Asn Met Met Ala Asp Ser Leu Asp Met Ala Leu
 50 55 60

Ser Asp Ala Glu Ser Tyr Pro Phe Phe Gly Asp Lys Arg Leu Val Tyr
 65 70 75 80

Ile Gln Asp Pro Phe Phe Leu Thr Gly Glu Lys Arg Lys Thr Asp Leu
 85 90 95

Asp His Asp Leu Asp Arg Leu Leu Ala Tyr Leu Gln Asn Pro Ala Asp
 100 105 110

Phe Thr Val Leu Val Phe Phe Ala Pro Tyr Glu Lys Leu Asp Lys Arg
115 120 125

Lys Lys Val Thr Lys Ala Leu Leu Gln Glu Ala Glu Ile Ile Asp Ala
130 135 140

Ser Ser Pro Asp Gln Arg Asp Leu Lys Asp Met Val Gln Lys Lys Val
145 150 155 160

Lys Ala Arg Gly Tyr Gln Phe Asp Lys Gly Ala Leu Lys Ala Leu Val
165 170 175

Glu Lys Thr Asn Ala Asn Leu Ser Arg Val Met Gln Glu Leu Asp Lys
180 185 190

Leu Phe Leu Tyr His Leu Asp Asp Lys Ile Ile Thr Val Gln Ser Val
195 200 205

Asp Gln Val Val Ser Pro Ser Leu Glu Ser Asn Val Phe Ser Ile Asn
210 215 220

Asp Tyr Ile Leu Ser Gly Gln Ser Gln Ala Ala Ile Arg Ala Phe Asn
225 230 235 240

Asp Leu Ile Gln Gln Lys Glu Glu Pro Ile Lys Ile Ile Ala Ile Met
245 250 255

Met Asn Gln Phe Arg Leu Leu Leu Gln Val Lys Ile Leu Arg Thr Lys
260 265 270

Gly Tyr Gln Gln Gly Glu Ile Ala Lys Ile Leu Lys Val His Pro Tyr
275 280 285

Arg Val Lys Leu Ala Ile Glu Lys Gln Glu Ile Phe Ser Lys Gln Ser
290 295 300

Leu Ser Thr Ala Tyr Arg Tyr Leu Ile Glu Ser Asp His Leu Ile Lys
305 310 315 320

Thr Gly Lys Val Thr Ser Gln Leu Gln Phe Glu Leu Phe Ala Leu Gln
325 330 335

Phe Lys Asp Ser Val Met Asn
340

<210> 81

<211> 477

<212> DNA

<213> *Alloioioccus otitidis*

<220>

<221> CDS

<222> (1)..(477)

<223>

<400> 81

atg	aat	cgc	gca	atc	tat	gca	ggc	agt	ttt	gat	ccg	att	acc	ctg	ggc	48
Met	Asn	Arg	Ala	Ile	Tyr	Ala	Gly	Ser	Phe	Asp	Pro	Ile	Thr	Leu	Gly	
1			5					10						15		

cac	ctg	gat	atc	att	aaa	agg	gcc	agc	cac	tta	ttc	gat	gaa	gtc	atc	96
His	Leu	Asp	Ile	Ile	Lys	Arg	Ala	Ser	His	Leu	Phe	Asp	Glu	Val	Ile	
			20					25					30			

gtt	gca	ggt	gct	aat	aat	aca	tcg	aaa	aat	agt	atg	ttg	aac	ttt	gac	144
Val	Ala	Val	Ala	Asn	Asn	Thr	Ser	Lys	Asn	Ser	Met	Leu	Asn	Phe	Asp	
			35					40				45				

caa	aaa	ttg	aac	ctg	ggt	gaa	caa	tca	att	gct	agc	cag	ggt	cta	gct	192
Gln	Lys	Leu	Asn	Leu	Val	Glu	Gln	Ser	Ile	Ala	Ser	Gln	Gly	Leu	Ala	
	50					55				60						

aat	ggt	caa	gcc	aag	aca	tta	gag	tca	ggc	ttg	att	ggt	gac	ttt	gct	240
Asn	Val	Gln	Ala	Lys	Thr	Leu	Glu	Ser	Gly	Leu	Ile	Val	Asp	Phe	Ala	
65					70				75					80		

aag	gac	caa	gga	gct	agt	agt	ctg	ggt	agg	ggg	ttg	cgg	tcg	ggt	aaa	288
Lys	Asp	Gln	Gly	Ala	Ser	Ser	Leu	Val	Arg	Gly	Leu	Arg	Ser	Val	Lys	
			85					90						95		

gac	ttt	gaa	tat	gag	att	gcc	att	gag	gac	tta	aat	aag	gtc	caa	gac	336
Asp	Phe	Glu	Tyr	Glu	Ile	Ala	Ile	Glu	Asp	Leu	Asn	Lys	Val	Gln	Asp	
			100					105					110			

cca	gct	att	gaa	aca	ggt	tac	cta	gtc	tcg	tct	tcc	aaa	tac	cgg	tcc	384
Pro	Ala	Ile	Glu	Thr	Val	Tyr	Leu	Val	Ser	Ser	Ser	Lys	Tyr	Arg	Ser	
			115				120					125				

att	tct	tcc	tct	att	ggt	cgg	gaa	att	att	aag	ttt	aat	ggc	cgg	ctt	432
Ile	Ser	Ser	Ser	Ile	Val	Arg	Glu	Ile	Ile	Lys	Phe	Asn	Gly	Arg	Leu	
			130			135				140						

gat	gac	cta	gta	cct	gac	ccc	gtc	gtc	gaa	tat	ttt	aaa	aaa	taa		477
Asp	Asp	Leu	Val	Pro	Asp	Pro	Val	Val	Glu	Tyr	Phe	Lys	Lys			
145					150				155							

<210> 82
<211> 158
<212> PRT
<213> Alloiococcus otitidis

<400> 82
Met Asn Arg Ala Ile Tyr Ala Gly Ser Phe Asp Pro Ile Thr Leu Gly
1 5 10 15

His Leu Asp Ile Ile Lys Arg Ala Ser His Leu Phe Asp Glu Val Ile
20 25 30

Val Ala Val Ala Asn Asn Thr Ser Lys Asn Ser Met Leu Asn Phe Asp
35 40 45

Gln Lys Leu Asn Leu Val Glu Gln Ser Ile Ala Ser Gln Gly Leu Ala
50 55 60

Asn Val Gln Ala Lys Thr Leu Glu Ser Gly Leu Ile Val Asp Phe Ala
65 70 75 80

Lys Asp Gln Gly Ala Ser Ser Leu Val Arg Gly Leu Arg Ser Val Lys
85 90 95

Asp Phe Glu Tyr Glu Ile Ala Ile Glu Asp Leu Asn Lys Val Gln Asp
100 105 110

Pro Ala Ile Glu Thr Val Tyr Leu Val Ser Ser Ser Lys Tyr Arg Ser
115 120 125

Ile Ser Ser Ser Ile Val Arg Glu Ile Ile Lys Phe Asn Gly Arg Leu
130 135 140

Asp Asp Leu Val Pro Asp Pro Val Val Glu Tyr Phe Lys Lys
145 150 155

<210> 83
<211> 1260
<212> DNA
<213> Alloiococcus otitidis

<220>
<221> CDS
<222> (28)..(1260)
<223>

<400> 83

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tca ggt ggc gca aag att aag gtt att ggt gtt ggc ggt gct ggt ggc	102
Ser Gly Gly Ala Lys Ile Lys Val Ile Gly Val Gly Gly Ala Gly Gly	
10 15 20 25	
aat gcc gtt aac cgg atg att gaa gat gga gtc gaa ggc gtt gaa ttt	150
Asn Ala Val Asn Arg Met Ile Glu Asp Gly Val Glu Gly Val Glu Phe	
30 35 40	
att gta gcc aat aca gat gtc caa gcc ctt gat gcc aac cga gct gag	198
Ile Val Ala Asn Thr Asp Val Gln Ala Leu Asp Ala Asn Arg Ala Glu	
45 50 55	
act aaa att caa ctc gga gag aag tta acc agg gga ctc ggt gcc gga	246
Thr Lys Ile Gln Leu Gly Glu Lys Leu Thr Arg Gly Leu Gly Ala Gly	
60 65 70	
gct aat cca gaa gtt ggc cgt aag tcg gct gaa gag agt gaa gaa acc	294
Ala Asn Pro Glu Val Gly Arg Lys Ser Ala Glu Glu Ser Glu Glu Thr	
75 80 85	
att gcc gaa gct ctt gaa gga gct gac atg gtc ttc gtt act gct ggt	342
Ile Ala Glu Ala Leu Glu Gly Ala Asp Met Val Phe Val Thr Ala Gly	
90 95 100 105	
atg ggt ggc ggt act ggt act ggc ggg gcg ggc att att gcc cgc att	390
Met Gly Gly Gly Thr Gly Thr Gly Gly Ala Gly Ile Ile Ala Arg Ile	
110 115 120	
gcc aaa gaa caa ggg gct ttg act gta ggg gtt att acc cgg ccg ttc	438
Ala Lys Glu Gln Gly Ala Leu Thr Val Gly Val Ile Thr Arg Pro Phe	
125 130 135	
act ttt gaa gga cca aaa cgt ggg cgc ttt gca gcc gaa ggg att gcc	486
Thr Phe Glu Gly Pro Lys Arg Gly Arg Phe Ala Ala Glu Gly Ile Ala	
140 145 150	
caa atg cgg gaa cat gtt gac acc ctt gtc acc atc tcc aac aac cgc	534
Gln Met Arg Glu His Val Asp Thr Leu Val Thr Ile Ser Asn Asn Arg	
155 160 165	
ttg cta gaa att gtg gac aag aaa aca ccg atg atg gaa gcc ttc aga	582
Leu Leu Glu Ile Val Asp Lys Lys Thr Pro Met Met Glu Ala Phe Arg	
170 175 180 185	
gaa gca gat aat gtc ctc cgc caa ggg gtt caa ggt ata tct gac ttg	630
Glu Ala Asp Asn Val Leu Arg Gln Gly Val Gln Gly Ile Ser Asp Leu	
190 195 200	
att acc aat cca ggc tac gtc aac tta gac ttt gcc gat gtc aaa acg	678
Ile Thr Asn Pro Gly Tyr Val Asn Leu Asp Phe Ala Asp Val Lys Thr	
205 210 215	
gtg atg gcc aac caa ggt tct gcc ttg atg ggg att ggg tct gct tca	726

Val Met Ala Asn Gln Gly Ser Ala Leu Met Gly Ile Gly Ser Ala Ser	
220 225 230	
ggt gag aat aga acg gct gaa gct act aag aaa gct att tca tct cca	774
Gly Glu Asn Arg Thr Ala Glu Ala Thr Lys Lys Ala Ile Ser Ser Pro	
235 240 245	
ctt ttg gaa gtc tcc ctc aat ggg gct gaa aat gtc cta tta aac ata	822
Leu Leu Glu Val Ser Leu Asn Gly Ala Glu Asn Val Leu Leu Asn Ile	
250 255 260 265	
acc gga aac caa gac tta acc ctc ttt gaa gct caa gat gct tct gat	870
Thr Gly Asn Gln Asp Leu Thr Leu Phe Glu Ala Gln Asp Ala Ser Asp	
270 275 280	
atc gtc ggg gct gct gct tct ggt gat gtt aat att atc ttc ggt act	918
Ile Val Gly Ala Ala Ala Ser Gly Asp Val Asn Ile Ile Phe Gly Thr	
285 290 295	
tcc atc aat gaa gac ctg gaa gat gag gtc atc gtt acc gtt att gca	966
Ser Ile Asn Glu Asp Leu Glu Asp Glu Val Ile Val Thr Val Ile Ala	
300 305 310	
act ggt atc act ggt aaa gac atg ggc gag aaa tct tct aaa tcc tca	1014
Thr Gly Ile Thr Gly Lys Asp Met Gly Glu Lys Ser Ser Lys Ser Ser	
315 320 325	
aac cgt agc caa ggt cct agt caa aaa agt caa gct cga tca gct agt	1062
Asn Arg Ser Gln Gly Pro Ser Gln Lys Ser Gln Ala Arg Ser Ala Ser	
330 335 340 345	
gag tct agc ttc tct agc tgg caa aac caa tcc aat gaa aga cca ggg	1110
Glu Ser Ser Phe Ser Ser Trp Gln Asn Gln Ser Asn Glu Arg Pro Gly	
350 355 360	
gaa gac caa gac cga cca agc tct caa aga cgg gaa gtc gat cgg tcc	1158
Glu Asp Gln Asp Arg Pro Ser Ser Gln Arg Arg Glu Val Asp Arg Ser	
365 370 375	
gaa aac ctg ttc aat gac gat agt aag gac cag cca gca gac tct ggt	1206
Glu Asn Leu Phe Asn Asp Asp Ser Lys Asp Gln Pro Ala Asp Ser Gly	
380 385 390	
gat gat gac gaa ttg gat acc cct cct ttc ttt aga cgt cgc cgc aag	1254
Asp Asp Asp Glu Leu Asp Thr Pro Pro Phe Phe Arg Arg Arg Arg Lys	
395 400 405	
aat tag	1260
Asn	
410	

<210> 84

<211> 410

<212> PRT

<213> Alloiococcus otitidis

<400> 84

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Val Ile Gly Val Gly Gly Ala Gly Gly Asn Ala Val Asn Arg Met Ile
 20 25 30

Glu Asp Gly Val Glu Gly Val Glu Phe Ile Val Ala Asn Thr Asp Val
 35 40 45

Gln Ala Leu Asp Ala Asn Arg Ala Glu Thr Lys Ile Gln Leu Gly Glu
 50 55 60

Lys Leu Thr Arg Gly Leu Gly Ala Gly Ala Asn Pro Glu Val Gly Arg
 65 70 75 80

Lys Ser Ala Glu Glu Ser Glu Glu Thr Ile Ala Glu Ala Leu Glu Gly
 85 90 95

Ala Asp Met Val Phe Val Thr Ala Gly Met Gly Gly Gly Thr Gly Thr
 100 105 110

Gly Gly Ala Gly Ile Ile Ala Arg Ile Ala Lys Glu Gln Gly Ala Leu
 115 120 125

Thr Val Gly Val Ile Thr Arg Pro Phe Thr Phe Glu Gly Pro Lys Arg
 130 135 140

Gly Arg Phe Ala Ala Glu Gly Ile Ala Gln Met Arg Glu His Val Asp
 145 150 155 160

Thr Leu Val Thr Ile Ser Asn Asn Arg Leu Leu Glu Ile Val Asp Lys
 165 170 175

Lys Thr Pro Met Met Glu Ala Phe Arg Glu Ala Asp Asn Val Leu Arg
 180 185 190

Gln Gly Val Gln Gly Ile Ser Asp Leu Ile Thr Asn Pro Gly Tyr Val
 195 200 205

Asn Leu Asp Phe Ala Asp Val Lys Thr Val Met Ala Asn Gln Gly Ser
 210 215 220

Ala Leu Met Gly Ile Gly Ser Ala Ser Gly Glu Asn Arg Thr Ala Glu
225 230 235 240

Ala Thr Lys Lys Ala Ile Ser Ser Pro Leu Leu Glu Val Ser Leu Asn
245 250 255

Gly Ala Glu Asn Val Leu Leu Asn Ile Thr Gly Asn Gln Asp Leu Thr
260 265 270

Leu Phe Glu Ala Gln Asp Ala Ser Asp Ile Val Gly Ala Ala Ala Ser
275 280 285

Gly Asp Val Asn Ile Ile Phe Gly Thr Ser Ile Asn Glu Asp Leu Glu
290 295 300

Asp Glu Val Ile Val Thr Val Ile Ala Thr Gly Ile Thr Gly Lys Asp
305 310 315 320

Met Gly Glu Lys Ser Ser Lys Ser Ser Asn Arg Ser Gln Gly Pro Ser
325 330 335

Gln Lys Ser Gln Ala Arg Ser Ala Ser Glu Ser Ser Phe Ser Ser Trp
340 345 350

Gln Asn Gln Ser Asn Glu Arg Pro Gly Glu Asp Gln Asp Arg Pro Ser
355 360 365

Ser Gln Arg Arg Glu Val Asp Arg Ser Glu Asn Leu Phe Asn Asp Asp
370 375 380

Ser Lys Asp Gln Pro Ala Asp Ser Gly Asp Asp Asp Glu Leu Asp Thr
385 390 395 400

Pro Pro Phe Phe Arg Arg Arg Arg Lys Asn
405 410

<210> 85

<211> 1377

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (13) .. (1377)

<223>

<400> 85
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ggg gtt tat aca agc ctt gat att gga acc act tca ata aaa gta gtt 99
Gly Val Tyr Thr Ser Leu Asp Ile Gly Thr Thr Ser Ile Lys Val Val
15 20 25

gtc agt gaa gtt gat aat aat cag ctc aaa gtt att gga gta gga aaa 147
Val Ser Glu Val Asp Asn Asn Gln Leu Lys Val Ile Gly Val Gly Lys
30 35 40 45

gct caa tca aaa ggt tta aaa agg ggc atg gtt gtc gat ata gat gct 195
Ala Gln Ser Lys Gly Leu Lys Arg Gly Met Val Val Asp Ile Asp Ala
50 55 60

acc gtc cag gcc att cat act gca gtg aag cag gct gct gat aag act 243
Thr Val Gln Ala Ile His Thr Ala Val Lys Gln Ala Ala Asp Lys Thr
65 70 75

ggg gtt atg atc aac cag ctc att gtt gga gtt cct gct aat ggt gtt 291
Gly Val Met Ile Asn Gln Leu Ile Val Gly Val Pro Ala Asn Gly Val
80 85 90

agt att gaa ccc tgt cac ggg gtc att act gta gat gac cgg tcc aag 339
Ser Ile Glu Pro Cys His Gly Val Ile Thr Val Asp Asp Arg Ser Lys
95 100 105

gaa ata gac agc cag gaa gtg aac cgg gta gtc aac cag tcc att gct 387
Glu Ile Asp Ser Gln Glu Val Asn Arg Val Val Asn Gln Ser Ile Ala
110 115 120 125

aat atc gtt ccg cca gat aga gac tta tta tcc gtc agt tta gaa gaa 435
Asn Ile Val Pro Pro Asp Arg Asp Leu Leu Ser Val Ser Leu Glu Glu
130 135 140

ttt att gta gat ggt ttt gat gaa att cat gat ccg aga ggc atg gtg 483
Phe Ile Val Asp Gly Phe Asp Glu Ile His Asp Pro Arg Gly Met Val
145 150 155

ggc cag cgg tta gaa ctt tac ggg aca gca att tca gtg cct aaa aca 531
Gly Gln Arg Leu Glu Leu Tyr Gly Thr Ala Ile Ser Val Pro Lys Thr
160 165 170

att tta cat aac att aga cgt tgt gtt gaa aaa gcg ggc tat caa att 579
Ile Leu His Asn Ile Arg Arg Cys Val Glu Lys Ala Gly Tyr Gln Ile
175 180 185

gct gcc tta att ctc cag ccc caa gcc atg gcc aag gta gcc ttg tct 627
Ala Ala Leu Ile Leu Gln Pro Gln Ala Met Ala Lys Val Ala Leu Ser
190 195 200 205

gag gat gag cgg aat ttt ggt aca gtt atg gtg gat ata ggc gga ggt 675
Glu Asp Glu Arg Asn Phe Gly Thr Val Met Val Asp Ile Gly Gly Gly
210 215 220

caa acg acc cta tca gcc att cac gat gag caa gtg aag tat gcc aat 723
Gln Thr Thr Leu Ser Ala Ile His Asp Glu Gln Val Lys Tyr Ala Asn
225 230 235

gtg gtc caa gaa gcc gga gaa tat att acc aaa gac att tcc att gtc 771
Val Val Gln Glu Ala Gly Glu Tyr Ile Thr Lys Asp Ile Ser Ile Val
240 245 250

atc aac acc tca cag caa aat gca gaa aag ctc aaa aga gaa gtt ggg 819
Ile Asn Thr Ser Gln Gln Asn Ala Glu Lys Leu Lys Arg Glu Val Gly
255 260 265

gcc att aaa agt cag tct gat tca act gtt caa gta gat gtt gta ggt 867
Ala Ile Lys Ser Gln Ser Asp Ser Thr Val Gln Val Asp Val Val Gly
270 275 280 285

caa aat gaa cct gtg aag att aaa gaa tcc tat gtc ggt gaa att att 915
Gln Asn Glu Pro Val Lys Ile Lys Glu Ser Tyr Val Gly Glu Ile Ile
290 295 300

gaa gcc cgg gtt agc caa atc ttt gaa aaa gtg aag gct gac ctt gac 963
Glu Ala Arg Val Ser Gln Ile Phe Glu Lys Val Lys Ala Asp Leu Asp
305 310 315

cca att aac gcc ttc caa ttg cca ggt ggt gcc gtt att tcc ggc ggt 1011
Pro Ile Asn Ala Phe Gln Leu Pro Gly Gly Ala Val Ile Ser Gly Gly
320 325 330

tca gct gcc ata cca ggt att gac agc ttg gct gaa gac atc ttc aag 1059
Ser Ala Ala Ile Pro Gly Ile Asp Ser Leu Ala Glu Asp Ile Phe Lys
335 340 345

gtt cgg tca gag ctc tac att ccc gac tac atg ggt atc cga act ccc 1107
Val Arg Ser Glu Leu Tyr Ile Pro Asp Tyr Met Gly Ile Arg Thr Pro
350 355 360 365

gcc ttc act gtg gca gtc ggc ttg acc ctc tac caa gcc cag act tct 1155
Ala Phe Thr Val Ala Val Gly Leu Thr Leu Tyr Gln Ala Gln Thr Ser
370 375 380

gat att gag cgg gcc atc aac cag tcc atc ttg caa aat atc ggt att 1203
Asp Ile Glu Arg Ala Ile Asn Gln Ser Ile Leu Gln Asn Ile Gly Ile
385 390 395

aat cca gat agc cag cct gct aac cgg ata gtt gac cag gat gat tca 1251
Asn Pro Asp Ser Gln Pro Ala Asn Arg Ile Val Asp Gln Asp Asp Ser
400 405 410

gtc caa agt cag gac caa aag acg caa gat gag cca gca gga gac caa 1299
Val Gln Ser Gln Asp Gln Lys Thr Gln Asp Glu Pro Ala Gly Asp Gln
415 420 425

gct agt cag tcg gat agt cca gaa gaa ggc aat ttt aca gac aga atc 1347
Ala Ser Gln Ser Asp Ser Pro Glu Glu Gly Asn Phe Thr Asp Arg Ile
430 435 440 445

1377

aag cat ttc ttt act aca ttt ttc gat taa
 Lys His Phe Phe Thr Thr Phe Phe Asp
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<210> 86
 <211> 454
 <212> PRT
 <213> Alloiococcus otitidis

<400> 86
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 20 25 30

Val Asp Asn Asn Gln Leu Lys Val Ile Gly Val Gly Lys Ala Gln Ser
 35 40 45

Lys Gly Leu Lys Arg Gly Met Val Val Asp Ile Asp Ala Thr Val Gln
 50 55 60

Ala Ile His Thr Ala Val Lys Gln Ala Ala Asp Lys Thr Gly Val Met
 65 70 75 80

Ile Asn Gln Leu Ile Val Gly Val Pro Ala Asn Gly Val Ser Ile Glu
 85 90 95

Pro Cys His Gly Val Ile Thr Val Asp Asp Arg Ser Lys Glu Ile Asp
 100 105 110

Ser Gln Glu Val Asn Arg Val Val Asn Gln Ser Ile Ala Asn Ile Val
 115 120 125

Pro Pro Asp Arg Asp Leu Leu Ser Val Ser Leu Glu Glu Phe Ile Val
 130 135 140

Asp Gly Phe Asp Glu Ile His Asp Pro Arg Gly Met Val Gly Gln Arg
 145 150 155 160

Leu Glu Leu Tyr Gly Thr Ala Ile Ser Val Pro Lys Thr Ile Leu His
 165 170 175

Asn Ile Arg Arg Cys Val Glu Lys Ala Gly Tyr Gln Ile Ala Ala Leu
 180 185 190

Ile Leu Gln Pro Gln Ala Met Ala Lys Val Ala Leu Ser Glu Asp Glu
195 200 205

Arg Asn Phe Gly Thr Val Met Val Asp Ile Gly Gly Gly Gln Thr Thr
210 215 220

Leu Ser Ala Ile His Asp Glu Gln Val Lys Tyr Ala Asn Val Val Gln
225 230 235 240

Glu Ala Gly Glu Tyr Ile Thr Lys Asp Ile Ser Ile Val Ile Asn Thr
245 250 255

Ser Gln Gln Asn Ala Glu Lys Leu Lys Arg Glu Val Gly Ala Ile Lys
260 265 270

Ser Gln Ser Asp Ser Thr Val Gln Val Asp Val Val Gly Gln Asn Glu
275 280 285

Pro Val Lys Ile Lys Glu Ser Tyr Val Gly Glu Ile Ile Glu Ala Arg
290 295 300

Val Ser Gln Ile Phe Glu Lys Val Lys Ala Asp Leu Asp Pro Ile Asn
305 310 315 320

Ala Phe Gln Leu Pro Gly Gly Ala Val Ile Ser Gly Gly Ser Ala Ala
325 330 335

Ile Pro Gly Ile Asp Ser Leu Ala Glu Asp Ile Phe Lys Val Arg Ser
340 345 350

Glu Leu Tyr Ile Pro Asp Tyr Met Gly Ile Arg Thr Pro Ala Phe Thr
355 360 365

Val Ala Val Gly Leu Thr Leu Tyr Gln Ala Gln Thr Ser Asp Ile Glu
370 375 380

Arg Ala Ile Asn Gln Ser Ile Leu Gln Asn Ile Gly Ile Asn Pro Asp
385 390 395 400

Ser Gln Pro Ala Asn Arg Ile Val Asp Gln Asp Asp Ser Val Gln Ser
405 410 415

Gln Asp Gln Lys Thr Gln Asp Glu Pro Ala Gly Asp Gln Ala Ser Gln
 420 425 430

Ser Asp Ser Pro Glu Glu Gly Asn Phe Thr Asp Arg Ile Lys His Phe
 435 440 445

Phe Thr Thr Phe Phe Asp
 450

<210> 87

<211> 1179

<212> DNA

<213> *Alloiococcus otitidis*

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<222> (16)..(1179)

<223>

<400> 87

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tca ggc ggt gga aca ggt ggc cat atc tac cca gcc ttg gcc ctt gct	99
Ser Gly Gly Gly Thr Gly Gly His Ile Tyr Pro Ala Leu Ala Leu Ala	
15 20 25	
aag cac cta gct agc tta cac tca gat gtc gag ttt ttg tat gtt ggc	147
Lys His Leu Ala Ser Leu His Ser Asp Val Glu Phe Leu Tyr Val Gly	
30 35 40	
act caa agg gga ttg gaa aat aaa ttg gtc ccc caa gca gga ctt gac	195
Thr Gln Arg Gly Leu Glu Asn Lys Leu Val Pro Gln Ala Gly Leu Asp	
45 50 55 60	
ttt atc ccg atc aaa gta gaa gga ttt agc cgg aag ttt aac ttc aaa	243
Phe Ile Pro Ile Lys Val Glu Gly Phe Ser Arg Lys Phe Asn Phe Lys	
65 70 75	
agc att aaa tat aat act aaa agt ctg att tat ttt cta aag gcc ctg	291
Ser Ile Lys Tyr Asn Thr Lys Ser Leu Ile Tyr Phe Leu Lys Ala Leu	
80 85 90	
agt aag tct aag caa atc atc aaa gac ttt cag cca gat gtg gta ata	339
Ser Lys Ser Lys Gln Ile Ile Lys Asp Phe Gln Pro Asp Val Val Ile	
95 100 105	
ggg aca ggt ggt tat gtt tgt gcc cct gtc ata tac cag gcg acc aag	387
Gly Thr Gly Gly Tyr Val Cys Ala Pro Val Ile Tyr Gln Ala Thr Lys	
110 115 120	
tta ggc att cca agt ctc att cac gaa caa aat agt gtc gcc ggg gtg	435

1107

gca aaa aga aac aag atg gcc caa caa gcg aaa gaa atg ggc caa c
Ala Lys Arg Asn Lys Met Ala Gln Gln Ala Lys Glu Met Gly Gln P

350 355 360

caa gct tca gac aag ttg atc gct ctc atc ttg tcc atg gtt aag gaa 1155
 Gln Ala Ser Asp Lys Leu Ile Ala Leu Ile Leu Ser Met Val Lys Glu
 365 370 375 380

gat att aac tca gac atc gat taa 1179
 Asp Ile Asn Ser Asp Ile Asp
 385

<210> 88
 <211> 387
 <212> PRT
 <213> *Alloiococcus otitidis*

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 Met Glu Thr Lys Lys Gln Ala Leu Lys Val Leu Leu Ser Gly Gly Gly
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 20 25 30

Ser Leu His Ser Asp Val Glu Phe Leu Tyr Val Gly Thr Gln Arg Gly
 35 40 45

Leu Glu Asn Lys Leu Val Pro Gln Ala Gly Leu Asp Phe Ile Pro Ile
 50 55 60

Lys Val Glu Gly Phe Ser Arg Lys Phe Asn Phe Lys Ser Ile Lys Tyr
 65 70 75 80

Asn Thr Lys Ser Leu Ile Tyr Phe Leu Lys Ala Leu Ser Lys Ser Lys
 85 90 95

Gln Ile Ile Lys Asp Phe Gln Pro Asp Val Val Ile Gly Thr Gly Gly
 100 105 110

Tyr Val Cys Ala Pro Val Ile Tyr Gln Ala Thr Lys Leu Gly Ile Pro
 115 120 125

Ser Leu Ile His Glu Gln Asn Ser Val Ala Gly Val Thr Asn Lys Phe
 130 135 140

Leu Ala Arg Tyr Val Asp Lys Ile Ala Leu Ser Phe Gln Glu Ala Glu
 145 150 155 160

Lys Ser Phe Ala Lys Tyr Lys Asp Lys Leu Val Leu Thr Gly Asn Pro
165 170 175

Arg Gly Gln Glu Val Ser Gln Val Lys Gly Gly Leu Ser Leu His Lys
180 185 190

Tyr Gly Met Asp Met Ser Gln Pro Ser Val Ile Ile Phe Gly Gly Ser
195 200 205

Arg Gly Ala Tyr Ala Ile Asn Lys Ala Phe Val Glu Ala Tyr Ser Gln
210 215 220

Leu Ala Glu Arg Asp Tyr Gln Val Leu Phe Val Pro Gly Ser Ala Asn
225 230 235 240

Phe Ser Arg Ile Lys Gln Glu Ile Asp Asn Arg Tyr Gly Gln His Lys
245 250 255

Pro Ser Asn Ile Phe Ile Glu Ser Tyr Ile Asp Asn Met Pro Gln Val
260 265 270

Phe Lys Ala Ile Asp Leu Val Val Cys Arg Ser Gly Ala Thr Thr Leu
275 280 285

Ala Glu Ile Met Ser Leu Gly Leu Ala Ser Ile Leu Ile Pro Ser Pro
290 295 300

Asn Val Thr Ala Asp His Gln Thr Lys Asn Ala Met Ser Leu Val Asn
305 310 315 320

Gln Gln Ala Gly Leu Met Ile Lys Glu Asn Asp Leu Asn Gly Gln Ser
325 330 335

Leu Leu Asn Cys Leu Asp Asp Leu Met His Asp Asp Ala Lys Arg Asn
340 345 350

Lys Met Ala Gln Gln Ala Lys Glu Met Gly Gln Pro Gln Ala Ser Asp
355 360 365

Lys Leu Ile Ala Leu Ile Leu Ser Met Val Lys Glu Asp Ile Asn Ser
370 375 380

Asp Ile Asp

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<222> (25)..(1428)  
<223>
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cct cat ata gca gtc att acc aat att tat tcc gcc cac ctt gac tac Pro His Ile Ala Val Ile Thr Asn Ile Tyr Ser Ala His Leu Asp Tyr 190 195 200	627
cat aag agt cgg gag gaa tat gtt agg gct aag cta agg att acc cag His Lys Ser Arg Glu Glu Tyr Val Arg Ala Lys Leu Arg Ile Thr Gln 205 210 215	675
gct caa ggt ccg gat gac tac cta gtc tac tac cag ggt cag gaa gaa Ala Gln Gly Pro Asp Asp Tyr Leu Val Tyr Tyr Gln Gly Gln Glu Glu 220 225 230	723
ttg gct agc ctg gtc aaa aaa tac tct aaa gcc cag ctg gtc ccc tat Leu Ala Ser Leu Val Lys Lys Tyr Ser Lys Ala Gln Leu Val Pro Tyr 235 240 245	771
act gac aag ggt caa ctg aac caa gga gcc tat atc aag gat gac tat Thr Asp Lys Gly Gln Leu Asn Gln Gly Ala Tyr Ile Lys Asp Asp Tyr 250 255 260 265	819
ctt atc tat aat caa gag cca gtc atg gct tta gac cga gtt caa gtt Leu Ile Tyr Asn Gln Glu Pro Val Met Ala Leu Asp Arg Val Gln Val 270 275 280	867
tct ggt agc cac aac tta caa aat att tta gca gct gtt tgc gta gct Ser Gly Ser His Asn Leu Gln Asn Ile Leu Ala Ala Val Cys Val Ala 285 290 295	915
aaa ata aag ggg ctc tct aac caa acc att gcc caa gct gtc aac cac Lys Ile Lys Gly Leu Ser Asn Gln Thr Ile Ala Gln Ala Val Asn His 300 305 310	963
ttc aaa ggg gtt gcc cac cgc agc cag gtg gtt ggg cgg tat gag gac Phe Lys Gly Val Ala His Arg Ser Gln Val Val Gly Arg Tyr Glu Asp 315 320 325	1011
cgg ctt ttt gtc aac gac tct aag gca acc aat agc ttg gcc aca cag Arg Leu Phe Val Asn Asp Ser Lys Ala Thr Asn Ser Leu Ala Thr Gln 330 335 340 345	1059
aag gca tta gaa gcc tat gac caa gat acc atc ttg tta gtg ggt ggc Lys Ala Leu Glu Ala Tyr Asp Gln Asp Thr Ile Leu Leu Val Gly Gly 350 355 360	1107
cta gac cgc caa gat gat ttt tcc aag ctt gac cat gct cta aac agg Leu Asp Arg Gln Asp Asp Phe Ser Lys Leu Asp His Ala Leu Asn Arg 365 370 375	1155
gtt aag ggg gtc gtt tgt ttt ggc cag acc aaa gat aag tta gcc cgg Val Lys Gly Val Val Cys Phe Gly Gln Thr Lys Asp Lys Leu Ala Arg 380 385 390	1203

tat ttt aaa gac cgt cac att gag ggt gtt gag ctt gcc cag aca gtt 1251
 Tyr Phe Lys Asp Arg His Ile Glu Gly Val Glu Leu Ala Gln Thr Val
 395 400 405
 cct gaa gca gtt gat ttg gct tac gac ttg agt gag cca gga caa gtc 1299
 Pro Glu Ala Val Asp Leu Ala Tyr Asp Leu Ser Glu Pro Gly Gln Val
 410 415 420 425
 att tta ttt tct cct gct tgt gca agt tgg gac caa tat gct aac ttt 1347
 Ile Leu Phe Ser Pro Ala Cys Ala Ser Trp Asp Gln Tyr Ala Asn Phe
 430 435 440
 gaa gag aga gga caa gat tat gtt gat gca atc cag cag ctg gtt gaa 1395
 Glu Glu Arg Gly Gln Asp Tyr Val Asp Ala Ile Gln Gln Leu Val Glu
 445 450 455
 aga cta gag caa agg agc aag tat gga aac taa 1428
 Arg Leu Glu Gln Arg Ser Lys Tyr Gly Asn
 460 465

<210> 90
 <211> 467
 <212> PRT
 <213> Alloioococcus otitidis

<400> 90
 Met Val Asp Ser Val Phe Cys Asn Lys Lys Val Leu Val Leu Gly Leu
 1 5 10 15

Ala Lys Ser Gly Leu Ser Ala Ala His Leu Leu Lys Lys Leu Gly Ala
 20 25 30

Lys Val Ile Val Asn Asp Lys Leu Ala Leu Glu Asn Asn Thr Glu Ala
 35 40 45

Gln Val Leu Ile Glu Glu Gly Phe Gln Val Ile Thr Gly Tyr His Pro
 50 55 60

Glu Asp Leu Leu Asp Ala Ser Phe Asp Phe Val Val Lys Asn Pro Gly
 65 70 75 80

Ile Pro Tyr Thr Asn Pro Val Val Gly Gln Ala Glu Lys Leu Ala Ile
 85 90 95

Pro Ile Leu Thr Glu Val Asp Val Ala Gly Ser Ile Leu Lys Ala Lys
 100 105 110

Pro Ile Ala Val Thr Gly Thr Asn Gly Lys Thr Thr Thr Val Ser Leu
 115 120 125

Ile Tyr Asp Ile Leu Ala Gln Asp Gln Ala Glu Ser Pro Glu Pro Lys
130 135 140

Pro Val Tyr Lys Leu Gly Asn Ile Gly Gln Pro Val Ser Asp Leu Ala
145 150 155 160

Leu Glu Ile Lys Ala Glu Ser Asn Leu Val Val Glu Leu Ser Ser Phe
165 170 175

Gln Leu Gln Ser Leu Thr Tyr Phe Thr Pro His Ile Ala Val Ile Thr
180 185 190

Asn Ile Tyr Ser Ala His Leu Asp Tyr His Lys Ser Arg Glu Glu Tyr
195 200 205

Val Arg Ala Lys Leu Arg Ile Thr Gln Ala Gln Gly Pro Asp Asp Tyr
210 215 220

Leu Val Tyr Tyr Gln Gly Gln Glu Glu Leu Ala Ser Leu Val Lys Lys
225 230 235 240

Tyr Ser Lys Ala Gln Leu Val Pro Tyr Thr Asp Lys Gly Gln Leu Asn
245 250 255

Gln Gly Ala Tyr Ile Lys Asp Asp Tyr Leu Ile Tyr Asn Gln Glu Pro
260 265 270

Val Met Ala Leu Asp Arg Val Gln Val Ser Gly Ser His Asn Leu Gln
275 280 285

Asn Ile Leu Ala Ala Val Cys Val Ala Lys Ile Lys Gly Leu Ser Asn
290 295 300

Gln Thr Ile Ala Gln Ala Val Asn His Phe Lys Gly Val Ala His Arg
305 310 315 320

Ser Gln Val Val Gly Arg Tyr Glu Asp Arg Leu Phe Val Asn Asp Ser
325 330 335

Lys Ala Thr Asn Ser Leu Ala Thr Gln Lys Ala Leu Glu Ala Tyr Asp
340 345 350

Gln Asp Thr Ile Leu Leu Val Gly Gly Leu Asp Arg Gln Asp Asp Phe
 355 360 365

Ser Lys Leu Asp His Ala Leu Asn Arg Val Lys Gly Val Val Cys Phe
 370 375 380

Gly Gln Thr Lys Asp Lys Leu Ala Arg Tyr Phe Lys Asp Arg His Ile
 385 390 395 400

Glu Gly Val Glu Leu Ala Gln Thr Val Pro Glu Ala Val Asp Leu Ala
 405 410 415

Tyr Asp Leu Ser Glu Pro Gly Gln Val Ile Leu Phe Ser Pro Ala Cys
 420 425 430

Ala Ser Trp Asp Gln Tyr Ala Asn Phe Glu Glu Arg Gly Gln Asp Tyr
 435 440 445

Val Asp Ala Ile Gln Gln Leu Val Glu Arg Leu Glu Gln Arg Ser Lys
 450 455 460

Tyr Gly Asn
 465

<210> 91
 <211> 651
 <212> DNA
 <213> *Alloiococcus otitidis*

<220>
 <221> CDS
 <222> (7)..(651)
 <223>

<400> 91
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 Met Lys Gln Lys Thr Gln Ala Thr Ala Val Asn Gln Thr Gln
 1 5 10

aca gag gca gaa gaa aga caa gaa acc cgt cgg aaa att ggc ctc atg 96
 Thr Glu Ala Glu Glu Arg Gln Glu Thr Arg Arg Lys Ile Gly Leu Met
 15 20 25 30

ggg ggg acc ttt aat ccg ccc cat ctg ggt cat tta ctg gta gct gaa 144
 Gly Gly Thr Phe Asn Pro Pro His Leu Gly His Leu Leu Val Ala Glu
 35 40 45

caa gtt tat gag gcc ttg gac ttg gat aat att cac ttt atg ccc act 192

Gln Val Tyr Glu Ala Leu Asp Leu Asp Asn Ile His Phe Met Pro Thr
50 55 60

gca aag ccg ggc cat gcc gct ggt aag gaa acc ata gat gcc tct tac 240
Ala Lys Pro Gly His Ala Ala Gly Lys Glu Thr Ile Asp Ala Ser Tyr
65 70 75

cgg gtt gat atg gtg gat tat gcc atc gaa gat aac ccc cac ttt tct 288
Arg Val Asp Met Val Asp Tyr Ala Ile Glu Asp Asn Pro His Phe Ser
80 85 90

ctt aac ttg act gaa gtg aac cgg gga ggg aca act tac acc atc gat 336
Leu Asn Leu Thr Glu Val Asn Arg Gly Gly Thr Thr Tyr Thr Ile Asp
95 100 105 110

acc att aaa gaa ttg aaa gag gct agc ccg aat aca gat tat tac ttc 384
Thr Ile Lys Glu Leu Lys Glu Ala Ser Pro Asn Thr Asp Tyr Tyr Phe
115 120 125

att att ggt gag gat tca gtt atg gat ttg gcc cag tgg aag aat att 432
Ile Ile Gly Glu Asp Ser Val Met Asp Leu Ala Gln Trp Lys Asn Ile
130 135 140

gaa caa tta ctg gat tta gtt caa ttt gtt ggt gtg aag cga cca ggc 480
Glu Gln Leu Leu Asp Leu Val Gln Phe Val Gly Val Lys Arg Pro Gly
145 150 155

tac caa gct gat gtg gac ttt ccc att att tgg gtg gat acg cca gaa 528
Tyr Gln Ala Asp Val Asp Phe Pro Ile Ile Trp Val Asp Thr Pro Glu
160 165 170

cta gat att agt tca agt gac atc agg caa agg gtg gca gaa ggg caa 576
Leu Asp Ile Ser Ser Ser Asp Ile Arg Gln Arg Val Ala Glu Gly Gln
175 180 185 190

tcc att aaa tat ttg acc cca gat agg gta aga gat tat att gaa gac 624
Ser Ile Lys Tyr Leu Thr Pro Asp Arg Val Arg Asp Tyr Ile Glu Asp
195 200 205

aat ggc tta tat aag ggt gaa gaa taa 651
Asn Gly Leu Tyr Lys Gly Glu Glu
210

<210> 92

<211> 214

<212> PRT

<213> Alloiococcus otitidis

<400> 92

Met Lys Gln Lys Thr Gln Ala Thr Ala Val Asn Gln Thr Gln Thr Glu
1 5 10 15

Ala Glu Glu Arg Gln Glu Thr Arg Arg Lys Ile Gly Leu Met Gly Gly
20 25 30

Thr Phe Asn Pro Pro His Leu Gly His Leu Leu Val Ala Glu Gln Val
35 40 45

Tyr Glu Ala Leu Asp Leu Asp Asn Ile His Phe Met Pro Thr Ala Lys
50 55 60

Pro Gly His Ala Ala Gly Lys Glu Thr Ile Asp Ala Ser Tyr Arg Val
65 70 75 80

Asp Met Val Asp Tyr Ala Ile Glu Asp Asn Pro His Phe Ser Leu Asn
85 90 95

Leu Thr Glu Val Asn Arg Gly Gly Thr Thr Tyr Thr Ile Asp Thr Ile
100 105 110

Lys Glu Leu Lys Glu Ala Ser Pro Asn Thr Asp Tyr Tyr Phe Ile Ile
115 120 125

Gly Glu Asp Ser Val Met Asp Leu Ala Gln Trp Lys Asn Ile Glu Gln
130 135 140

Leu Leu Asp Leu Val Gln Phe Val Gly Val Lys Arg Pro Gly Tyr Gln
145 150 155 160

Ala Asp Val Asp Phe Pro Ile Ile Trp Val Asp Thr Pro Glu Leu Asp
165 170 175

Ile Ser Ser Ser Asp Ile Arg Gln Arg Val Ala Glu Gly Gln Ser Ile
180 185 190

Lys Tyr Leu Thr Pro Asp Arg Val Arg Asp Tyr Ile Glu Asp Asn Gly
195 200 205

Leu Tyr Lys Gly Glu Glu
210

<210> 93

<211> 666

<212> DNA

<213> *Alloiococcus otitidis*

<220>

<221> CDS

<222> (1)..(666)

<223>

<400> 93

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Met Val Gly Gly Leu Ile Phe Val Leu Thr Ala Ser Asn Lys Arg Lys	
1 5 10 15	
gga agt ttg tcc atg acc tat ttg tta ggc cta acc ggt ggc att gcc	96
Gly Ser Leu Ser Met Thr Tyr Leu Leu Gly Leu Thr Gly Gly Ile Ala	
20 25 30	
agt ggg aag tct act gtt agc cag gtt ttt aag gaa aag ggt atc caa	144
Ser Gly Lys Ser Thr Val Ser Gln Val Phe Lys Glu Lys Gly Ile Gln	
35 40 45	
gtg gtt gat gct gac cga gtt gcc cga cag gtt gtt gaa cct gga agt	192
Val Val Asp Ala Asp Arg Val Ala Arg Gln Val Val Glu Pro Gly Ser	
50 55 60	
cca ggc tta gac cag ctt gtt gat tat ttt ggc cag gag att ttg acc	240
Pro Gly Leu Asp Gln Leu Val Asp Tyr Phe Gly Gln Glu Ile Leu Thr	
65 70 75 80	
cag gat ggg ggc ttg gac cgc aaa tat tta ggc gac ctt atc ttc cgg	288
Gln Asp Gly Gly Leu Asp Arg Lys Tyr Leu Gly Asp Leu Ile Phe Arg	
85 90 95	
aat agc cag gcc aag gag gct gtc aac cgg atc ctc cac cct ttg att	336
Asn Ser Gln Ala Lys Glu Ala Val Asn Arg Ile Leu His Pro Leu Ile	
100 105 110	
agg cag tct atc caa aat caa att aaa act gcc ata ggc caa gac ttg	384
Arg Gln Ser Ile Gln Asn Gln Ile Lys Thr Ala Ile Gly Gln Asp Leu	
115 120 125	
gat ttg tta gtt tta gac atc ccc ctc ctt tac gag aca ggt cag gca	432
Asp Leu Leu Val Leu Asp Ile Pro Leu Leu Tyr Glu Thr Gly Gln Ala	
130 135 140	
gac gac tac cag gcc gtc atg gtg gtt tcg ctt ccc tac cag gac cag	480
Asp Asp Tyr Gln Ala Val Met Val Val Ser Leu Pro Tyr Gln Asp Gln	
145 150 155 160	
gtg agt cgg tta atg gac cgg gat ggg att gac cga gac caa gcc ctg	528
Val Ser Arg Leu Met Asp Arg Asp Gly Ile Asp Arg Asp Gln Ala Leu	
165 170 175	
cgc aag att cag gcc caa atg tca ttg gaa gaa aaa gtg aag ttg gcg	576
Arg Lys Ile Gln Ala Gln Met Ser Leu Glu Glu Lys Val Lys Leu Ala	
180 185 190	
gac tat gtc att gat aac agc gga agc aag gaa gaa agc cgt cag cag	624
Asp Tyr Val Ile Asp Asn Ser Gly Ser Lys Glu Glu Ser Arg Gln Gln	
195 200 205	
gtt gaa gct tgg ttg gat caa aag ggt ttt aaa aac ttg taa	666
Val Glu Ala Trp Leu Asp Gln Lys Gly Phe Lys Asn Leu	

210

215

220

<210> 94

<211> 221

<212> PRT

<213> Alloiococcus otitidis

<400> 94

Met Val Gly Gly Leu Ile Phe Val Leu Thr Ala Ser Asn Lys Arg Lys
1 5 10 15

Gly Ser Leu Ser Met Thr Tyr Leu Leu Gly Leu Thr Gly Gly Ile Ala
20 25 30

Ser Gly Lys Ser Thr Val Ser Gln Val Phe Lys Glu Lys Gly Ile Gln
35 40 45

Val Val Asp Ala Asp Arg Val Ala Arg Gln Val Val Glu Pro Gly Ser
50 55 60

Pro Gly Leu Asp Gln Leu Val Asp Tyr Phe Gly Gln Glu Ile Leu Thr
65 70 75 80

Gln Asp Gly Gly Leu Asp Arg Lys Tyr Leu Gly Asp Leu Ile Phe Arg
85 90 95

Asn Ser Gln Ala Lys Glu Ala Val Asn Arg Ile Leu His Pro Leu Ile
100 105 110

Arg Gln Ser Ile Gln Asn Gln Ile Lys Thr Ala Ile Gly Gln Asp Leu
115 120 125

Asp Leu Leu Val Leu Asp Ile Pro Leu Leu Tyr Glu Thr Gly Gln Ala
130 135 140

Asp Asp Tyr Gln Ala Val Met Val Val Ser Leu Pro Tyr Gln Asp Gln
145 150 155 160

Val Ser Arg Leu Met Asp Arg Asp Gly Ile Asp Arg Asp Gln Ala Leu
165 170 175

Arg Lys Ile Gln Ala Gln Met Ser Leu Glu Glu Lys Val Lys Leu Ala
180 185 190

Asp Tyr Val Ile Asp Asn Ser Gly Ser Lys Glu Glu Ser Arg Gln Gln
 195 200 205

Val Glu Ala Trp Leu Asp Gln Lys Gly Phe Lys Asn Leu
 210 215 220

<210> 95
 <211> 1335
 <212> DNA
 <213> Alloiococcus otitidis

<220>
 <221> CDS
 <222> (4)..(1335)
 <223>

<400> 95
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 Met Asp Gln Asp Thr Ile Tyr His Phe Val Gly Ile Lys Gly Ser
 1 5 10 15
 ggc atg agt tca ctt gcc act atc ttg ttt gac aag ggc tta aat gtc 96
 Gly Met Ser Ser Leu Ala Thr Ile Leu Phe Asp Lys Gly Leu Asn Val
 20 25 30
 caa gga tct gat gtc aaa aag tat ttc ttt acc caa aaa agc tta gaa 144
 Gln Gly Ser Asp Val Lys Lys Tyr Phe Phe Thr Gln Lys Ser Leu Glu
 35 40 45
 gaa aaa aat ata aac att tta gaa ttt gac cct gat aac atc aaa cca 192
 Glu Lys Asn Ile Asn Ile Leu Glu Phe Asp Pro Asp Asn Ile Lys Pro
 50 55 60
 ggt atg acc ctg ata gca ggc aat gcc ttt gga gac aac cat ccc gag 240
 Gly Met Thr Leu Ile Ala Gly Asn Ala Phe Gly Asp Asn His Pro Glu
 65 70 75
 ctg gtc cga ggt cga gag ctc ggt tta gaa atc atc cgc tac cat gat 288
 Leu Val Arg Gly Arg Glu Leu Gly Leu Glu Ile Ile Arg Tyr His Asp
 80 85 90 95
 ttt atc ggt gac ctt atc gaa cac ttt act tcc atc gct att acc ggg 336
 Phe Ile Gly Asp Leu Ile Glu His Phe Thr Ser Ile Ala Ile Thr Gly
 100 105 110
 tct cac ggt aag acc tcc aca act ggt ttg atg gcc cat gtt ttc tcc 384
 Ser His Gly Lys Thr Ser Thr Thr Gly Leu Met Ala His Val Phe Ser
 115 120 125
 ggt att gat agc acc tcc tac tta att gga gat ggg acc ggc cat ggg 432
 Gly Ile Asp Ser Thr Ser Tyr Leu Ile Gly Asp Gly Thr Gly His Gly
 130 135 140
 gaa aaa ggt gcc aag tat ttt gtc ttg gaa gcc tgc gaa tac aag cgg 480
 Glu Lys Gly Ala Lys Tyr Phe Val Leu Glu Ala Cys Glu Tyr Lys Arg

145	150	155	
cac ttt ttg gcc tac cga ccg gac tat gcg gtt atg acc aat att gac			528
His Phe Leu Ala Tyr Arg Pro Asp Tyr Ala Val Met Thr Asn Ile Asp			
160	165	170	175
ttt gac cac ccg gac tat tac aag tct att gaa gat gtc caa gtg gcc			576
Phe Asp His Pro Asp Tyr Tyr Lys Ser Ile Glu Asp Val Gln Val Ala			
180	185	190	
ttt gat gaa ttc agc cac cag gtc aaa aaa tac ctc ttt gcc tgc ggg			624
Phe Asp Glu Phe Ser His Gln Val Lys Lys Tyr Leu Phe Ala Cys Gly			
195	200	205	
gac gac caa cgt ctt cgg cag gtc aaa gcc cag gtg ccg gtc att tac			672
Asp Asp Gln Arg Leu Arg Gln Val Lys Ala Gln Val Pro Val Ile Tyr			
210	215	220	
tac ggt cta aat gaa gac aat gac ttt gtg gct aaa aac atc gac cga			720
Tyr Gly Leu Asn Glu Asp Asn Asp Phe Val Ala Lys Asn Ile Asp Arg			
225	230	235	
agt cgt gaa ggg tct gcc ttc gac ctt tat att aag gga gaa ttt tac			768
Ser Arg Glu Gly Ser Ala Phe Asp Leu Tyr Ile Lys Gly Glu Phe Tyr			
240	245	250	255
aaa cac ttc acc atc cca acc tat ggc aac cac aat att caa aat gcc			816
Lys His Phe Thr Ile Pro Thr Tyr Gly Asn His Asn Ile Gln Asn Ala			
260	265	270	
ttg gcg gtt ata gca gta gct tac tac gaa ggg tta gac caa gat ttg			864
Leu Ala Val Ile Ala Val Ala Tyr Tyr Glu Gly Leu Asp Gln Asp Leu			
275	280	285	
gtt gcc caa aga ttg gct aat ttt gct ggg gtg aaa cgc cgg ttt acc			912
Val Ala Gln Arg Leu Ala Asn Phe Ala Gly Val Lys Arg Arg Phe Thr			
290	295	300	
gag aag gtg gtc ggg gac act act att atc gat gac tat gct cac cac			960
Glu Lys Val Val Gly Asp Thr Thr Ile Ile Asp Asp Tyr Ala His His			
305	310	315	
cct gct gaa ata agg gca acg att gat gcg gcc cgg caa aaa tac ccg			1008
Pro Ala Glu Ile Arg Ala Thr Ile Asp Ala Ala Arg Gln Lys Tyr Pro			
320	325	330	335
gac aag gac att gtg acg gtc ttc cag ccc cac acc ttt acc cgg aca			1056
Asp Lys Asp Ile Val Thr Val Phe Gln Pro His Thr Phe Thr Arg Thr			
340	345	350	
gtc gcc ctc cta gat gaa ttt gcc cag gcc ttg gac ttg gca gac cag			1104
Val Ala Leu Leu Asp Glu Phe Ala Gln Ala Leu Asp Leu Ala Asp Gln			
355	360	365	
gtt tac ttg tgt gat atc ttt aat tca gct aga gaa aag tca ggc gat			1152
Val Tyr Leu Cys Asp Ile Phe Asn Ser Ala Arg Glu Lys Ser Gly Asp			
370	375	380	

att tcc atc caa gat ctt ttg gct aaa acc agc aag gcc gac cag gtg 1200
 Ile Ser Ile Gln Asp Leu Leu Ala Lys Thr Ser Lys Ala Asp Gln Val
 385 390 395

att gag gaa gac gat gtg tct cct ctg ctt gac caa cat ggg caa gtg 1248
 Ile Glu Glu Asp Asp Val Ser Pro Leu Leu Asp Gln His Gly Gln Val
 400 405 410 415

att att ttc atg gga gca gga gac atc agc aag ttt gaa aaa gcc tat 1296
 Ile Ile Phe Met Gly Ala Gly Asp Ile Ser Lys Phe Glu Lys Ala Tyr
 420 425 430

gaa agc ttg ttg agc tca acc tac cac tcc cag gtc taa 1335
 Glu Ser Leu Leu Ser Ser Thr Tyr His Ser Gln Val
 435 440

<210> 96

<211> 443

<212> PRT

<213> Alloiococcus otitidis

<400> 96

Met Asp Gln Asp Thr Ile Tyr His Phe Val Gly Ile Lys Gly Ser Gly
 1 5 10 15

Met Ser Ser Leu Ala Thr Ile Leu Phe Asp Lys Gly Leu Asn Val Gln
 20 25 30

Gly Ser Asp Val Lys Lys Tyr Phe Phe Thr Gln Lys Ser Leu Glu Glu
 35 40 45

Lys Asn Ile Asn Ile Leu Glu Phe Asp Pro Asp Asn Ile Lys Pro Gly
 50 55 60

Met Thr Leu Ile Ala Gly Asn Ala Phe Gly Asp Asn His Pro Glu Leu
 65 70 75 80

Val Arg Gly Arg Glu Leu Gly Leu Glu Ile Ile Arg Tyr His Asp Phe
 85 90 95

Ile Gly Asp Leu Ile Glu His Phe Thr Ser Ile Ala Ile Thr Gly Ser
 100 105 110

His Gly Lys Thr Ser Thr Thr Gly Leu Met Ala His Val Phe Ser Gly
 115 120 125

Ile Asp Ser Thr Ser Tyr Leu Ile Gly Asp Gly Thr Gly His Gly Glu

130

135

140

Lys Gly Ala Lys Tyr Phe Val Leu Glu Ala Cys Glu Tyr Lys Arg His
145 150 155 160

Phe Leu Ala Tyr Arg Pro Asp Tyr Ala Val Met Thr Asn Ile Asp Phe
165 170 175

Asp His Pro Asp Tyr Tyr Lys Ser Ile Glu Asp Val Gln Val Ala Phe
180 185 190

Asp Glu Phe Ser His Gln Val Lys Lys Tyr Leu Phe Ala Cys Gly Asp
195 200 205

Asp Gln Arg Leu Arg Gln Val Lys Ala Gln Val Pro Val Ile Tyr Tyr
210 215 220

Gly Leu Asn Glu Asp Asn Asp Phe Val Ala Lys Asn Ile Asp Arg Ser
225 230 235 240

Arg Glu Gly Ser Ala Phe Asp Leu Tyr Ile Lys Gly Glu Phe Tyr Lys
245 250 255

His Phe Thr Ile Pro Thr Tyr Gly Asn His Asn Ile Gln Asn Ala Leu
260 265 270

Ala Val Ile Ala Val Ala Tyr Tyr Glu Gly Leu Asp Gln Asp Leu Val
275 280 285

Ala Gln Arg Leu Ala Asn Phe Ala Gly Val Lys Arg Arg Phe Thr Glu
290 295 300

Lys Val Val Gly Asp Thr Thr Ile Ile Asp Asp Tyr Ala His His Pro
305 310 315 320

Ala Glu Ile Arg Ala Thr Ile Asp Ala Ala Arg Gln Lys Tyr Pro Asp
325 330 335

Lys Asp Ile Val Thr Val Phe Gln Pro His Thr Phe Thr Arg Thr Val
340 345 350

Ala Leu Leu Asp Glu Phe Ala Gln Ala Leu Asp Leu Ala Asp Gln Val
355 360 365

Tyr Leu Cys Asp Ile Phe Asn Ser Ala Arg Glu Lys Ser Gly Asp Ile
 370 375 380

Ser Ile Gln Asp Leu Leu Ala Lys Thr Ser Lys Ala Asp Gln Val Ile
 385 390 395 400

Glu Glu Asp Asp Val Ser Pro Leu Leu Asp Gln His Gly Gln Val Ile
 405 410 415

Ile Phe Met Gly Ala Gly Asp Ile Ser Lys Phe Glu Lys Ala Tyr Glu
 420 425 430

Ser Leu Leu Ser Ser Thr Tyr His Ser Gln Val
 435 440

<210> 97

<211> 1050

<212> DNA

<213> Alloiococcus otitidis

<220>

<221> CDS

<222> (19)..(1050)

<223>

<400> 97

acaaaattat ttacgtgt atg gag gaa tta ata gtg cca tta tta gac tta 51
 Met Glu Glu Leu Ile Val Pro Leu Leu Asp Leu
 1 5 10

aat gac cat gac cgc gtt cag gaa tat gag gac ttt gtc caa aac cac 99
 Asn Asp His Asp Arg Val Gln Glu Tyr Glu Asp Phe Val Gln Asn His
 15 20 25

ccc cag ggc cac ctg atg cag tct acc aaa tgg atc cag gtt aag gaa 147
 Pro Gln Gly His Leu Met Gln Ser Thr Lys Trp Ile Gln Val Lys Glu
 30 35 40

ggc tgg gac ggt gac tat gtt tac ctt acc gat gac caa gac cgg atc 195
 Gly Trp Asp Gly Asp Tyr Val Tyr Leu Thr Asp Asp Gln Asp Arg Ile
 45 50 55

aag gca tgc ttg tcc att cta tca gtc aaa aat gac gga gaa cat gcc 243
 Lys Ala Cys Leu Ser Ile Leu Ser Val Lys Asn Asp Gly Glu His Ala
 60 65 70 75

ttc tta tat gcg cca aga ggg ccg gtt tgt gac ttt cat gat aca gac 291
 Phe Leu Tyr Ala Pro Arg Gly Pro Val Cys Asp Phe His Asp Thr Asp
 80 85 90

ttg gtg acc gac tta att aag gaa gcc caa gtc gta gcg gac aag cac Leu Val Thr Asp Leu Ile Lys Glu Ala Gln Val Val Ala Asp Lys His 95 100 105	339
aag gcc ttt ttg ttg cgg atg gac ccg gaa acc ctt cat gat cct gac Lys Ala Phe Leu Leu Arg Met Asp Pro Glu Thr Leu His Asp Pro Asp 110 115 120	387
ctg gtc gaa aaa tac cgc gat tta ggc tat act ttc cgg tca gct gag Leu Val Glu Lys Tyr Arg Asp Leu Gly Tyr Thr Phe Arg Ser Ala Glu 125 130 135	435
caa gaa gat gaa cac gtc ttc tcc aac ccc cgc ttc cac atg atg acg Gln Glu Asp Glu His Val Phe Ser Asn Pro Arg Phe His Met Met Thr 140 145 150 155	483
gac tta agg ggt cat gat gaa gaa agc ttg ctg atg gcc ttc acc agc Asp Leu Arg Gly His Asp Glu Glu Ser Leu Leu Met Ala Phe Thr Ser 160 165 170	531
aat aac cgg cgc aag atc cgc aaa act tac aaa aat aac ctc cag acc Asn Asn Arg Arg Lys Ile Arg Lys Thr Tyr Lys Asn Asn Leu Gln Thr 175 180 185	579
cac tat ctg acc gtg gat gat gag ggt tat gac cag gcc ttg gat gac His Tyr Leu Thr Val Asp Asp Glu Gly Tyr Asp Gln Ala Leu Asp Asp 190 195 200	627
ttt tat gaa ttg acc caa ata atg gca gaa cgg caa ggg att act cac Phe Tyr Glu Leu Thr Gln Ile Met Ala Glu Arg Gln Gly Ile Thr His 205 210 215	675
cgg ccc aaa gac tac ttt gac cgg tta atg cac agc ttt gag gat gct Arg Pro Lys Asp Tyr Phe Asp Arg Leu Met His Ser Phe Glu Asp Ala 220 225 230 235	723
aaa ttg ttc cag acc tac cac gaa gat gac ctc cta gct act tgt atc Lys Leu Phe Gln Thr Tyr His Glu Asp Asp Leu Leu Ala Thr Cys Ile 240 245 250	771
ttg gtg agc tat aat aaa aaa tcc ttc tac atg tat gca gct tct tcc Leu Val Ser Tyr Asn Lys Lys Ser Phe Tyr Met Tyr Ala Ala Ser Ser 255 260 265	819
aac aaa aaa cga aat tta aat ggg tct ttg caa gaa aat tac gaa gcc Asn Lys Lys Arg Asn Leu Asn Gly Ser Leu Gln Glu Asn Tyr Glu Ala 270 275 280	867
atg aag tat gcc ttg gcc cga gga agc gaa gaa tat gat atg ggt ggg Met Lys Tyr Ala Leu Ala Arg Gly Ser Glu Glu Tyr Asp Met Gly Gly 285 290 295	915
gtc ttt ggc ttt gac aag tcg gac ggc ctc tac cgg ttt aaa aaa atc Val Phe Gly Phe Asp Lys Ser Asp Gly Leu Tyr Arg Phe Lys Lys Ile 300 305 310 315	963
ttt acc ggt cat gaa ggg ctg aaa gaa ttt atg ggt gaa ttg gat gtg	1011

Phe Thr Gly His Glu Gly Leu Lys Glu Phe Met Gly Glu Leu Asp Val
 320 325 330

gtc tat gac caa gac cta tac gac gat ttt att tct taa
 Val Tyr Asp Gln Asp Leu Tyr Asp Asp Phe Ile Ser
 335 340

1050

<210> 98

<211> 343

<212> PRT

<213> Alloiococcus otitidis

<400> 98

Met Glu Glu Leu Ile Val Pro Leu Leu Asp Leu Asn Asp His Asp Arg
 1 5 10 15

Val Gln Glu Tyr Glu Asp Phe Val Gln Asn His Pro Gln Gly His Leu
 20 25 30

Met Gln Ser Thr Lys Trp Ile Gln Val Lys Glu Gly Trp Asp Gly Asp
 35 40 45

Tyr Val Tyr Leu Thr Asp Asp Gln Asp Arg Ile Lys Ala Cys Leu Ser
 50 55 60

Ile Leu Ser Val Lys Asn Asp Gly Glu His Ala Phe Leu Tyr Ala Pro
 65 70 75 80

Arg Gly Pro Val Cys Asp Phe His Asp Thr Asp Leu Val Thr Asp Leu
 85 90 95

Ile Lys Glu Ala Gln Val Val Ala Asp Lys His Lys Ala Phe Leu Leu
 100 105 110

Arg Met Asp Pro Glu Thr Leu His Asp Pro Asp Leu Val Glu Lys Tyr
 115 120 125

Arg Asp Leu Gly Tyr Thr Phe Arg Ser Ala Glu Gln Glu Asp Glu His
 130 135 140

Val Phe Ser Asn Pro Arg Phe His Met Met Thr Asp Leu Arg Gly His
 145 150 155 160

Asp Glu Glu Ser Leu Leu Met Ala Phe Thr Ser Asn Asn Arg Arg Lys
 165 170 175

Ile Arg Lys Thr Tyr Lys Asn Asn Leu Gln Thr His Tyr Leu Thr Val
 180 185 190

Asp Asp Glu Gly Tyr Asp Gln Ala Leu Asp Asp Phe Tyr Glu Leu Thr
 195 200 205

Gln Ile Met Ala Glu Arg Gln Gly Ile Thr His Arg Pro Lys Asp Tyr
 210 215 220

Phe Asp Arg Leu Met His Ser Phe Glu Asp Ala Lys Leu Phe Gln Thr
 225 230 235 240

Tyr His Glu Asp Asp Leu Leu Ala Thr Cys Ile Leu Val Ser Tyr Asn
 245 250 255

Lys Lys Ser Phe Tyr Met Tyr Ala Ala Ser Ser Asn Lys Lys Arg Asn
 260 265 270

Leu Asn Gly Ser Leu Gln Glu Asn Tyr Glu Ala Met Lys Tyr Ala Leu
 275 280 285

Ala Arg Gly Ser Glu Glu Tyr Asp Met Gly Gly Val Phe Gly Phe Asp
 290 295 300

Lys Ser Asp Gly Leu Tyr Arg Phe Lys Lys Ile Phe Thr Gly His Glu
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Gly Leu Lys Glu Phe Met Gly Glu Leu Asp Val Val Tyr Asp Gln Asp
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Leu Tyr Asp Asp Phe Ile Ser
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Leu	Ile	Met	Ala	Gly	Ala	Gly	Ser	Gly	Lys	Thr	Arg	Val	Leu	Thr	His	
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cgg	ata	gct	tac	ttg	atc	caa	gaa	aaa	ggg	gtt	aat	cct	tgg	aat	atc	195
Arg	Ile	Ala	Tyr	Leu	Ile	Gln	Glu	Lys	Gly	Val	Asn	Pro	Trp	Asn	Ile	
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Leu	Ala	Ile	Thr	Phe	Thr	Asn	Lys	Ala	Ala	Gly	Glu	Met	Lys	Asp	Arg	
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Phe	His	Ser	Met	Cys	Val	Arg	Ile	Leu	Arg	Arg	Asp	Gly	Asp	Gln	Ile	
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ggc	tat	aac	cgt	gcc	ttc	acc	att	gct	gac	cct	agt	gaa	cag	aaa	agt	387
Gly	Tyr	Asn	Arg	Ala	Phe	Thr	Ile	Ala	Asp	Pro	Ser	Glu	Gln	Lys	Ser	
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Val	Val	Ala	Asp	Cys	Tyr	Asp	Ala	Tyr	Gln	Arg	Gln	Leu	Arg	Gln	Ser	
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Glu	Ala	Met	Asp	Phe	Asp	Asp	Leu	Ile	Met	Gln	Thr	Val	Arg	Leu	Phe	
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Lys	Glu	Lys	Pro	Asp	Thr	Leu	Ser	Tyr	Tyr	Gln	Ala	Lys	Phe	Gln	Tyr	
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gct gac cag tct att tat ggt tgg cgg ggg gct gat atg gga aat att Ala Asp Gln Ser Ile Tyr Gly Trp Arg Gly Ala Asp Met Gly Asn Ile 255 260 265			819
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caa aat tac cgg tca acc aag tct ata atc agg gca gcc aat gat gtt Gln Asn Tyr Arg Ser Thr Lys Ser Ile Ile Arg Ala Ala Asn Asp Val 285 290 295			915
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gat gag ggg gac aag gtc agc tta tac gct gcc cgg agc gag cag gat Asp Glu Gly Asp Lys Val Ser Leu Tyr Ala Ala Arg Ser Glu Gln Asp 315 320 325 330			1011
gaa gcc cag ttt atc gta ggg acc atc cat gac cta aca gaa ggc aaa Glu Ala Gln Phe Ile Val Gly Thr Ile His Asp Leu Thr Glu Gly Lys 335 340 345			1059
aag gct ggc tat ggg gac atc gcc atc ctc tac cgg acc aat gcc atg Lys Ala Gly Tyr Gly Asp Ile Ala Ile Leu Tyr Arg Thr Asn Ala Met 350 355 360			1107
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atc gtc ggc gga acc ggc ttt tac caa aga aaa gaa atc cgt gac ctg Ile Val Gly Gly Thr Gly Phe Tyr Gln Arg Lys Glu Ile Arg Asp Leu 380 385 390			1203
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tca cgg atc gtt aat gag ccc aaa aga ggg att gga ccc ggc acc ctg Ser Arg Ile Val Asn Glu Pro Lys Arg Gly Ile Gly Pro Gly Thr Leu 415 420 425			1299
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aca gct ctc aat gcg gat gct acc aac ctg cct agt cgg gct gtc aac Thr Ala Leu Asn Ala Asp Ala Thr Asn Leu Pro Ser Arg Ala Val Asn 445 450 455			1395

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gaa tac tta ccg att act gat ttg acc gaa aaa atc tta gag gat act Glu Tyr Leu Pro Ile Thr Asp Leu Thr Glu Lys Ile Leu Glu Asp Thr 475 480 485 490	1491
ggc tac caa aaa gcc tta gaa aaa gac cgg act ctt gaa tct cag gca Gly Tyr Gln Lys Ala Leu Glu Lys Asp Arg Thr Leu Glu Ser Gln Ala 495 500 505	1539
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cag caa gaa gac gac aac aag tca ctc tta gcc ttc tta act gac ctt Gln Gln Glu Asp Asp Asn Lys Ser Leu Leu Ala Phe Leu Thr Asp Leu 525 530 535	1635
tcc tta ttg tca cca gct gat gat gtt gaa gag ggt cgg ggc cag gtc Ser Leu Leu Ser Pro Ala Asp Asp Val Glu Glu Gly Arg Gly Gln Val 540 545 550	1683
acc atg atg acc ctc cat gca gcc aag ggg ttg gaa ttc ccc tat gtc Thr Met Met Thr Leu His Ala Ala Lys Gly Leu Glu Phe Pro Tyr Val 555 560 565 570	1731
ttt atc gct ggt atg gaa gag gga atc ttc ccc ttg tcc cgg gcg gct Phe Ile Ala Gly Met Glu Glu Gly Ile Phe Pro Leu Ser Arg Ala Ala 575 580 585	1779
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gaa att tct tct gac ctg gtc caa gac ctt ggt gct aca act ggg tct Glu Ile Ser Ser Asp Leu Val Gln Asp Leu Gly Ala Thr Thr Gly Ser 635 640 645 650	1971
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Gly Ser Gly Lys Thr Arg Val Leu Thr His Arg Ile Ala Tyr Leu Ile
35 40 45

Gln Glu Lys Gly Val Asn Pro Trp Asn Ile Leu Ala Ile Thr Phe Thr
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Asn Lys Ala Ala Gly Glu Met Lys Asp Arg Val Gln Lys Leu Val Ser
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Gln Gly Gly Ser Gly Val Trp Val Ser Thr Phe His Ser Met Cys Val
85 90 95

Arg Ile Leu Arg Arg Asp Gly Asp Gln Ile Gly Tyr Asn Arg Ala Phe
100 105 110

Thr Ile Ala Asp Pro Ser Glu Gln Lys Ser Leu Met Lys Gln Val Leu
115 120 125

Lys Asp Leu Asn Ile Asp Pro Lys Arg Tyr Asn Pro Lys Ala Ile Leu
130 135 140

Ala Glu Ile Ser Asn Ala Lys Asn Asp Leu Leu Asp Glu Gln Thr Tyr
145 150 155 160

Arg Lys Gln Ala Asp Asp Tyr Phe Lys Glu Val Val Ala Asp Cys Tyr
165 170 175

Asp Ala Tyr Gln Arg Gln Leu Arg Gln Ser Glu Ala Met Asp Phe Asp
180 185 190

Asp Leu Ile Met Gln Thr Val Arg Leu Phe Lys Glu Lys Pro Asp Thr
195 200 205

Leu Ser Tyr Tyr Gln Ala Lys Phe Gln Tyr Ile His Val Asp Glu Tyr
210 215 220

Gln Asp Thr Asn Gln Ala Gln Tyr Gln Leu Val Gln Leu Leu Ala Gln
225 230 235 240

Arg Phe Lys Asn Val Cys Val Val Gly Asp Ala Asp Gln Ser Ile Tyr
245 250 255

Gly Trp Arg Gly Ala Asp Met Gly Asn Ile Leu Asn Phe Glu Lys Asp
260 265 270

Tyr Pro Glu Ala Gln Thr Ile Phe Leu Glu Gln Asn Tyr Arg Ser Thr
275 280 285

Lys Ser Ile Ile Arg Ala Ala Asn Asp Val Ile Gln Asn Asn Ile Asn
290 295 300

Arg Arg Asp Lys Asn Leu Trp Thr Ala Asn Asp Glu Gly Asp Lys Val
305 310 315 320

Ser Leu Tyr Ala Ala Arg Ser Glu Gln Asp Glu Ala Gln Phe Ile Val
325 330 335

Gly Thr Ile His Asp Leu Thr Glu Gly Lys Lys Ala Gly Tyr Gly Asp
340 345 350

Ile Ala Ile Leu Tyr Arg Thr Asn Ala Met Ser Arg Val Ile Glu Glu
355 360 365

Thr Phe Ile Lys Ser Asn Ile Pro Tyr Lys Ile Val Gly Gly Thr Gly
370 375 380

Phe Tyr Gln Arg Lys Glu Ile Arg Asp Leu Ile Ala Tyr Leu Thr Leu
385 390 395 400

Val Ala Asn Pro Ala Asp Asp Leu Ser Phe Ser Arg Ile Val Asn Glu
405 410 415

Pro Lys Arg Gly Ile Gly Pro Gly Thr Leu Asp Lys Leu Arg Gln Ala
420 425 430

Gly Gln Glu Met Gly Trp Ser Leu Tyr Glu Thr Ala Leu Asn Ala Asp
435 440 445

Ala Thr Asn Leu Pro Ser Arg Ala Val Asn Arg Leu Leu Asp Phe Ser
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Gln Met Ile Glu Asn Phe Arg Lys Met Thr Glu Tyr Leu Pro Ile Thr
465 470 475 480

Asp Leu Thr Glu Lys Ile Leu Glu Asp Thr Gly Tyr Gln Lys Ala Leu
485 490 495

Glu Lys Asp Arg Thr Leu Glu Ser Gln Ala Arg Leu Glu Asn Leu Gln
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Glu Phe Tyr Ser Val Thr Gln Glu Phe Asp Gln Gln Glu Asp Asp Asn
515 520 525

Lys Ser Leu Leu Ala Phe Leu Thr Asp Leu Ser Leu Leu Ser Pro Ala
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Asp Asp Val Glu Glu Gly Arg Gly Gln Val Thr Met Met Thr Leu His
545 550 555 560

Ala Ala Lys Gly Leu Glu Phe Pro Tyr Val Phe Ile Ala Gly Met Glu
565 570 575

Glu Gly Ile Phe Pro Leu Ser Arg Ala Ala Glu Asp Pro Glu Ser Leu
580 585 590

Glu Glu Glu Arg Arg Leu Ala Tyr Val Gly Ile Thr Arg Ala Glu Gln
 595 600 605

Ala Leu Tyr Leu Thr Arg Ala Met Met Arg Gln Leu Tyr Gly Arg Thr
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Gln Ala Asn Pro Lys Ser Arg Phe Leu Ser Glu Ile Ser Ser Asp Leu
 625 630 635 640

Val Gln Asp Leu Gly Ala Thr Thr Gly Ser Leu Ser Gln Thr Gly Gly
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Lys Val Ser Pro Arg Leu Gly Gly Arg Lys Ala Ser Gly Tyr Lys Ala
 660 665 670

Asn Ala Trp Ser Gln Gln Ser Val Gly Ala Thr Gly Ala Glu Lys Glu
 675 680 685

Asp Trp Glu Val Gly Asp Lys Val His His Lys Lys Trp Gly Gln Gly
 690 695 700

Thr Ile Ile Glu Ile Lys Gly Ser Gly Ser Asp Leu Gln Leu Asn Ile
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Ile Glu Lys Ile
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<212> DNA

<213> *Alloiococcus otitidis*

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aca gtc aag gta gaa ggg gct aag aat gct gcc ctt cct atc ctg gct 96
 Thr Val Lys Val Glu Gly Ala Lys Asn Ala Ala Leu Pro Ile Leu Ala

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Ala	Ser	Leu	Leu	Pro	Glu	Asp	Gly	Lys	Ser	His	Leu	Ser	Asn	Val	Pro	
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tta	cta	tct	gat	att	tac	acg	atg	caa	gaa	gtt	ttg	cgt	tac	tta	aac	192
Leu	Leu	Ser	Asp	Ile	Tyr	Thr	Met	Gln	Glu	Val	Leu	Arg	Tyr	Leu	Asn	.
		50					55					60				
gtt	gac	att	gac	ttc	gat	gaa	gac	cac	aac	gaa	atc	gtc	ata	gat	gct	240
Val	Asp	Ile	Asp	Phe	Asp	Glu	Asp	His	Asn	Glu	Ile	Val	Ile	Asp	Ala	
	65					70					75					
aca	gga	gac	ctg	aat	tcc	aat	acc	cct	tat	gaa	ttt	atg	agc	aag	atg	288
Thr	Gly	Asp	Leu	Asn	Ser	Asn	Thr	Pro	Tyr	Glu	Phe	Met	Ser	Lys	Met	
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cgg	gct	tcc	atc	att	gtc	atg	ggg	ccc	tta	cta	gcc	cgt	aat	ggg	tat	336
Arg	Ala	Ser	Ile	Ile	Val	Met	Gly	Pro	Leu	Leu	Ala	Arg	Asn	Gly	Tyr	
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gcc	aaa	gtc	gct	ctt	cct	ggg	ggg	tgc	gcg	att	ggg	act	cgt	cct	att	384
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gac	ttg	cac	tta	aaa	ggc	ttc	cgg	gct	atg	ggg	gtc	gat	gtg	gaa	gtc	432
Asp	Leu	His	Leu	Lys	Gly	Phe	Arg	Ala	Met	Gly	Val	Asp	Val	Glu	Val	
		130					135					140				
gaa	gga	ggg	tat	gtg	atc	gcc	aca	gtt	caa	gat	gaa	ctg	gat	ggc	gct	480
Glu	Gly	Gly	Tyr	Val	Ile	Ala	Thr	Val	Gln	Asp	Glu	Leu	Asp	Gly	Ala	
	145					150					155					
gat	att	tac	ctt	gac	ttc	cca	agt	gtt	gga	gct	aca	caa	aat	att	ttg	528
Asp	Ile	Tyr	Leu	Asp	Phe	Pro	Ser	Val	Gly	Ala	Thr	Gln	Asn	Ile	Leu	
160					165					170					175	
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Met	Ala	Ala	Thr	Arg	Ala	Lys	Gly	Thr	Thr	Val	Ile	Glu	Asn	Ala	Ala	
				180					185					190		
cga	gaa	cct	gaa	att	gtt	gac	ctt	gcc	aac	tat	ttg	aac	aag	atg	ggg	624
Arg	Glu	Pro	Glu	Ile	Val	Asp	Leu	Ala	Asn	Tyr	Leu	Asn	Lys	Met	Gly	
			195					200					205			
gcc	cgt	att	tac	ggg	gcc	gga	acc	aat	acc	atg	aga	att	gaa	ggg	gta	672
Ala	Arg	Ile	Tyr	Gly	Ala	Gly	Thr	Asn	Thr	Met	Arg	Ile	Glu	Gly	Val	
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Asp	Lys	Leu	Glu	Ala	Cys	Asp	His	Ser	Ile	Ile	Ala	Asp	Arg	Ile	Glu	
	225					230					235					
agt	ggc	acc	ttt	atg	gta	gca	gct	ggg	gtc	acc	caa	ggg	aat	gtc	ttg	768
Ser	Gly	Thr	Phe	Met	Val	Ala	Ala	Gly	Val	Thr	Gln	Gly	Asn	Val	Leu	
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cct ggc ttc cca act gat atg cag tca ccg atg aca gtc gcc caa acc Pro Gly Phe Pro Thr Asp Met Gln Ser Pro Met Thr Val Ala Gln Thr 305 310 315	960
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gac cgg ggc tac tat aaa ttt cac gaa aaa tta cag caa tta ggt gct Asp Arg Gly Tyr Tyr Lys Phe His Glu Lys Leu Gln Gln Leu Gly Ala 400 405 410 415	1248
tcc att gaa cga atc gac gag gaa att caa gtt gac cag gaa gcc agc Ser Ile Glu Arg Ile Asp Glu Glu Ile Gln Val Asp Gln Glu Ala Ser 420 425 430	1296
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<211> 436

<212> PRT

<213> Alloiococcus otitidis

<400> 102

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Asp Ile Asp Phe Asp Glu Asp His Asn Glu Ile Val Ile Asp Ala Thr	65	70	75
Gly Asp Leu Asn Ser Asn Thr Pro Tyr Glu Phe Met Ser Lys Met Arg	85	90	95
Ala Ser Ile Ile Val Met Gly Pro Leu Leu Ala Arg Asn Gly Tyr Ala	100	105	110
Lys Val Ala Leu Pro Gly Gly Cys Ala Ile Gly Thr Arg Pro Ile Asp	115	120	125
Leu His Leu Lys Gly Phe Arg Ala Met Gly Val Asp Val Glu Val Glu	130	135	140
Gly Gly Tyr Val Ile Ala Thr Val Gln Asp Glu Leu Asp Gly Ala Asp	145	150	155
Ile Tyr Leu Asp Phe Pro Ser Val Gly Ala Thr Gln Asn Ile Leu Met	165	170	175
Ala Ala Thr Arg Ala Lys Gly Thr Thr Val Ile Glu Asn Ala Ala Arg	180	185	190
Glu Pro Glu Ile Val Asp Leu Ala Asn Tyr Leu Asn Lys Met Gly Ala	195	200	205
Arg Ile Tyr Gly Ala Gly Thr Asn Thr Met Arg Ile Glu Gly Val Asp	210	215	220
Lys Leu Glu Ala Cys Asp His Ser Ile Ile Ala Asp Arg Ile Glu Ser	225	230	235
			240

Gly Thr Phe Met Val Ala Ala Gly Val Thr Gln Gly Asn Val Leu Ile
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Glu Asp Cys Ile Val Glu His Asn Arg Pro Leu Ile Ser Lys Leu Ser
260 265 270

Glu Met Gly Val Gln Phe Glu Glu Glu Lys Thr Gly Leu Arg Val Met
275 280 285

Gly Pro Glu Thr Leu Gln Ala Thr Asp Val Lys Thr Leu Pro Tyr Pro
290 295 300

Gly Phe Pro Thr Asp Met Gln Ser Pro Met Thr Val Ala Gln Thr Leu
305 310 315 320

Ala Glu Gly Arg Ser Ile Met Arg Glu Thr Val Phe Glu Asn Arg Phe
325 330 335

Met His Met Glu Glu Leu Arg Lys Met Asp Ala Gln Phe Thr Val Asp
340 345 350

Gly Gln Ser Leu Ile Ile Glu Gly Gly Lys Lys Leu Gln Gly Ala Arg
355 360 365

Val Gln Ser Ser Asp Leu Arg Ala Ser Ala Ser Leu Ile Ile Ala Gly
370 375 380

Leu Val Ala Asp Gly Val Thr Lys Val Thr Asn Leu Asn His Leu Asp
385 390 395 400

Arg Gly Tyr Tyr Lys Phe His Glu Lys Leu Gln Gln Leu Gly Ala Ser
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Lys Lys Gly Glu
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Gln Ala Gly Val Tyr Gln Leu Phe Asp Arg Ile Leu Ala Asn His Ala
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ctc aag cat gcc tat ctt ttt gaa ggt ttg gcc gga tca ggc aaa ctg      150
Leu Lys His Ala Tyr Leu Phe Glu Gly Leu Ala Gly Ser Gly Lys Leu
      25                      30                      35

gag atg agc cgg tat att gcc aag aga ctg ttt tgc ccc aac caa gac      198
Glu Met Ser Arg Tyr Ile Ala Lys Arg Leu Phe Cys Pro Asn Gln Asp
      40                      45                      50                      55

cag gga caa gct tgc caa gtt tgt ccc act tgc ttg cgc att gac cag      246
Gln Gly Gln Ala Cys Gln Val Cys Pro Thr Cys Leu Arg Ile Asp Gln
      60                      65                      70

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Gly Gln His Pro Asp Val Val Glu Ile Ala Pro Glu Gly Lys Gly Arg
      75                      80                      85

tcg att agg gta gac cgg gta cga cag gtc aag gat gcc cta agc aag      342
Ser Ile Arg Val Asp Arg Val Arg Gln Val Lys Asp Ala Leu Ser Lys
      90                      95                      100

tct ggt gtg gag agt caa aag aaa atg att atc ctt aac cag gct gat      390
Ser Gly Val Glu Ser Gln Lys Lys Met Ile Ile Leu Asn Gln Ala Asp
      105                      110                      115

aaa atg acc ccc agt gca gcc aac agc ctg ctt aaa ttt ctg gaa gag      438
Lys Met Thr Pro Ser Ala Ala Asn Ser Leu Leu Lys Phe Leu Glu Glu
      120                      125                      130                      135

ccg gca ggg gat gtg act att ttc ttg tta gtt act agc cgg caa aac      486
Pro Ala Gly Asp Val Thr Ile Phe Leu Leu Val Thr Ser Arg Gln Asn
      140                      145                      150

ctt ttg cca act att gtt tcc cgc tgc cag gtt atc cag ttt gcc aag      534
Leu Leu Pro Thr Ile Val Ser Arg Cys Gln Val Ile Gln Phe Ala Lys
      155                      160                      165

cag gat tta aag act cgg att gag gac tta gtg gaa gcc ggt ttg tcc      582
Gln Asp Leu Lys Thr Arg Ile Glu Asp Leu Val Glu Ala Gly Leu Ser
      170                      175                      180

cag gaa gaa gcc cac ttg gcc agc cac ctc agc caa gac tta gac ttg      630

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Gln	Glu	Glu	Ala	His	Leu	Ala	Ser	His	Leu	Ser	Gln	Asp	Leu	Asp	Leu	
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gct	aag	tcc	ctc	att	gag	gaa	gag	gac	ttg	ctg	gca	gtc	agt	caa	aaa	678
Ala	Lys	Ser	Leu	Ile	Glu	Glu	Glu	Asp	Leu	Leu	Ala	Val	Ser	Gln	Lys	
200					205				210					215		
att	tgg	cag	tgg	ttt	agc	tat	ctc	atg	aac	caa	gat	gac	ttg	gcc	ttt	726
Ile	Trp	Gln	Trp	Phe	Ser	Tyr	Leu	Met	Asn	Gln	Asp	Asp	Leu	Ala	Phe	
				220					225					230		
atc	cta	gtc	caa	aga	gac	tta	atg	gcc	ttt	atc	caa	gac	cgg	gat	gac	774
Ile	Leu	Val	Gln	Arg	Asp	Leu	Met	Ala	Phe	Ile	Gln	Asp	Arg	Asp	Asp	
			235					240					245			
tgc	cag	atg	gtt	tgt	gac	tta	atc	ctc	tac	ctc	ttc	caa	gac	ctg	ctc	822
Cys	Gln	Met	Val	Cys	Asp	Leu	Ile	Leu	Tyr	Leu	Phe	Gln	Asp	Leu	Leu	
	250						255					260				
cac	tta	cac	tac	cat	tta	gat	agt	ccg	gcc	tgc	ttc	gca	ggc	cac	gaa	870
His	Leu	His	Tyr	His	Leu	Asp	Ser	Pro	Ala	Cys	Phe	Ala	Gly	His	Glu	
	265					270					275					
agt	gac	ctc	cgc	tac	ttt	atg	gac	ctg	ctt	tcg	atc	aag	caa	gtg	tct	918
Ser	Asp	Leu	Arg	Tyr	Phe	Met	Asp	Leu	Leu	Ser	Ile	Lys	Gln	Val	Ser	
280					285					290					295	
tat	gcc	atg	caa	gcc	acc	ctg	caa	gct	aaa	aga	gaa	gtg	gac	cac	aat	966
Tyr	Ala	Met	Gln	Ala	Thr	Leu	Gln	Ala	Lys	Arg	Glu	Val	Asp	His	Asn	
				300					305					310		
gtg	gcc	agt	cag	gct	gtt	tta	gaa	ggc	ttg	act	ttg	gac	ttg	cag	gaa	1014
Val	Ala	Ser	Gln	Ala	Val	Leu	Glu	Gly	Leu	Thr	Leu	Asp	Leu	Gln	Glu	
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agt	ata	ggc	taa													1026
Ser	Ile	Gly														
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<212> PRT

<213> Alloiooccus otitidis

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Arg	Ile	Leu	Ala	Asn	His	Ala	Leu	Lys	His	Ala	Tyr	Leu	Phe	Glu	Gly
		20						25					30		

Leu	Ala	Gly	Ser	Gly	Lys	Leu	Glu	Met	Ser	Arg	Tyr	Ile	Ala	Lys	Arg
		35					40					45			

Leu Phe Cys Pro Asn Gln Asp Gln Gly Gln Ala Cys Gln Val Cys Pro
50 55 60

Thr Cys Leu Arg Ile Asp Gln Gly Gln His Pro Asp Val Val Glu Ile
65 70 75 80

Ala Pro Glu Gly Lys Gly Arg Ser Ile Arg Val Asp Arg Val Arg Gln
85 90 95

Val Lys Asp Ala Leu Ser Lys Ser Gly Val Glu Ser Gln Lys Lys Met
100 105 110

Ile Ile Leu Asn Gln Ala Asp Lys Met Thr Pro Ser Ala Ala Asn Ser
115 120 125

Leu Leu Lys Phe Leu Glu Glu Pro Ala Gly Asp Val Thr Ile Phe Leu
130 135 140

Leu Val Thr Ser Arg Gln Asn Leu Leu Pro Thr Ile Val Ser Arg Cys
145 150 155 160

Gln Val Ile Gln Phe Ala Lys Gln Asp Leu Lys Thr Arg Ile Glu Asp
165 170 175

Leu Val Glu Ala Gly Leu Ser Gln Glu Glu Ala His Leu Ala Ser His
180 185 190

Leu Ser Gln Asp Leu Asp Leu Ala Lys Ser Leu Ile Glu Glu Glu Asp
195 200 205

Leu Leu Ala Val Ser Gln Lys Ile Trp Gln Trp Phe Ser Tyr Leu Met
210 215 220

Asn Gln Asp Asp Leu Ala Phe Ile Leu Val Gln Arg Asp Leu Met Ala
225 230 235 240

Phe Ile Gln Asp Arg Asp Asp Cys Gln Met Val Cys Asp Leu Ile Leu
245 250 255

Tyr Leu Phe Gln Asp Leu Leu His Leu His Tyr His Leu Asp Ser Pro
260 265 270

Ala Cys Phe Ala Gly His Glu Ser Asp Leu Arg Tyr Phe Met Asp Leu
 275 280 285

Leu Ser Ile Lys Gln Val Ser Tyr Ala Met Gln Ala Thr Leu Gln Ala
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Lys Arg Glu Val Asp His Asn Val Ala Ser Gln Ala Val Leu Glu Gly
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 Ser Phe Ala Asp Val Ser Gly Gln His Val Val Thr Lys Thr Leu Lys
 15 20 25

aat gcc att aaa aat gat aat acc agt cat gcc tac ctg ttt act gga 147
 Asn Ala Ile Lys Asn Asp Asn Thr Ser His Ala Tyr Leu Phe Thr Gly
 30 35 40 45

ccc cgg ggg acg ggc aag acc agt gtg gca aaa ata ttt gcc aag gcc 195
 Pro Arg Gly Thr Gly Lys Thr Ser Val Ala Lys Ile Phe Ala Lys Ala
 50 55 60

att aat tgc ccc tac tcg gat gat ggg gag cct tgt aat gaa tgt cag 243
 Ile Asn Cys Pro Tyr Ser Asp Asp Gly Glu Pro Cys Asn Glu Cys Gln
 65 70 75

att tgc cag gag atc acc cag ggt agt cta ggc gat gtc atc gaa atc 291
 Ile Cys Gln Glu Ile Thr Gln Gly Ser Leu Gly Asp Val Ile Glu Ile
 80 85 90

gat gcg gcc agc aat aat ggg gtg gaa gag att cgc gat att agg gaa 339
 Asp Ala Ala Ser Asn Asn Gly Val Glu Glu Ile Arg Asp Ile Arg Glu
 95 100 105

aag gct aat tat gcc cca act tcg gcc gtt tac aag gtc tac att atc 387
 Lys Ala Asn Tyr Ala Pro Thr Ser Ala Val Tyr Lys Val Tyr Ile Ile

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gat gag gtc cat atg tta tcc tct ggg gcc ttt aac gcc ctc ttg aaa				435
Asp Glu Val His Met Leu Ser Ser Gly Ala Phe Asn Ala Leu Leu Lys	130	135	140	
aca ctg gaa gag cct cca gcc aat gtg gtc ttt atc tta gca acg act				483
Thr Leu Glu Glu Pro Pro Ala Asn Val Val Phe Ile Leu Ala Thr Thr	145	150	155	
gaa ccc cac aag att ccg gct acc att atc tcc cgg acc cag cgt ttt				531
Glu Pro His Lys Ile Pro Ala Thr Ile Ile Ser Arg Thr Gln Arg Phe	160	165	170	
gat ttt aag cgg att gac aac cag gac atc atc gac cgc ttg att tat				579
Asp Phe Lys Arg Ile Asp Asn Gln Asp Ile Ile Asp Arg Leu Ile Tyr	175	180	185	
atc tta gaa gaa gac cag gtc ccc tac agc aaa gaa gcc gtc cta agc				627
Ile Leu Glu Glu Asp Gln Val Pro Tyr Ser Lys Glu Ala Val Leu Ser	190	195	200	205
cta gcc aat gca gcg gaa ggt ggg atg cgg gat gcc ttg agt atg ttg				675
Leu Ala Asn Ala Ala Glu Gly Gly Met Arg Asp Ala Leu Ser Met Leu	210	215	220	
gac cag gcc tta agc ttt atg aca gat gag tta aca gaa gaa gtt gcc				723
Asp Gln Ala Leu Ser Phe Met Thr Asp Glu Leu Thr Glu Glu Val Ala	225	230	235	
ctc cag att aca ggg agc att acc cag tct ctc ttg ctt gaa tac ttg				771
Leu Gln Ile Thr Gly Ser Ile Thr Gln Ser Leu Leu Leu Glu Tyr Leu	240	245	250	
cag gtg att agc caa ggt cag acg gaa gaa gga ctc aag ctc ttg caa				819
Gln Val Ile Ser Gln Gly Gln Thr Glu Glu Gly Leu Lys Leu Leu Gln	255	260	265	
gaa gtt tta ggg gaa ggc aag gac cct agc cgg ttt gtg gaa gac gct				867
Glu Val Leu Gly Glu Gly Lys Asp Pro Ser Arg Phe Val Glu Asp Ala	270	275	280	285
att atg atg acc cgg gac ctc ttg ctt tac caa act agc caa ggc gat				915
Ile Met Met Thr Arg Asp Leu Leu Leu Tyr Gln Thr Ser Gln Gly Asp	290	295	300	
aat ttt gtt cct aaa ttg gct cgc tta gac gac cag ttt gaa gac ctg				963
Asn Phe Val Pro Lys Leu Ala Arg Leu Asp Asp Gln Phe Glu Asp Leu	305	310	315	
gcg aag gac ttg gac aag gag atg gcc tac cat att att gat gtc tta				1011
Ala Lys Asp Leu Asp Lys Glu Met Ala Tyr His Ile Ile Asp Val Leu	320	325	330	
aac caa acc caa gac gat ctc cgc cta agc aac cat ggg gaa gtc tat				1059
Asn Gln Thr Gln Asp Asp Leu Arg Leu Ser Asn His Gly Glu Val Tyr	335	340	345	

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acc atc cag gcc agc caa gtc aac atg gtg gac cag gat aat aaa gaa Thr Ile Gln Ala Ser Gln Val Asn Met Val Asp Gln Asp Asn Lys Glu 370 375 380	1155
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tca aaa gct ggc ccc aag caa tct ggc cct ggc aag tct aga agc cac Ser Lys Ala Gly Pro Lys Gln Ser Gly Pro Gly Lys Ser Arg Ser His 415 420 425	1299
cgt cac cag caa ggc ttc aag gtt aac cgg aaa gcc gtt tac tct atc Arg His Gln Gln Gly Phe Lys Val Asn Arg Lys Ala Val Tyr Ser Ile 430 435 440 445	1347
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cca gac ttg atc aat gtc ttg acc atc agt caa aag gct atc tta aac Pro Asp Leu Ile Asn Val Leu Thr Ile Ser Gln Lys Ala Ile Leu Asn 465 470 475	1443
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ctg gtc tgt gtg cct gaa gac aag tgg ccg act atc cgc cgc gat ttt Leu Val Cys Val Pro Glu Asp Lys Trp Pro Thr Ile Arg Arg Asp Phe 530 535 540	1635
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agt gac ggc aag tcg gat gat gac cca ggt caa gaa gac aac cag gcc Ser Asp Gly Lys Ser Asp Asp Asp Pro Gly Gln Glu Asp Asn Gln Ala 560 565 570	1731

ctt aac aag gct gtg gag ctt ttc ggt aaa gac aat att aca atc aaa 1779
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gat taa 1785
Asp
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35 40 45

Thr Gly Lys Thr Ser Val Ala Lys Ile Phe Ala Lys Ala Ile Asn Cys
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Pro Tyr Ser Asp Asp Gly Glu Pro Cys Asn Glu Cys Gln Ile Cys Gln
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Glu Ile Thr Gln Gly Ser Leu Gly Asp Val Ile Glu Ile Asp Ala Ala
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Ser Asn Asn Gly Val Glu Glu Ile Arg Asp Ile Arg Glu Lys Ala Asn
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Tyr Ala Pro Thr Ser Ala Val Tyr Lys Val Tyr Ile Ile Asp Glu Val
115 120 125

His Met Leu Ser Ser Gly Ala Phe Asn Ala Leu Leu Lys Thr Leu Glu
130 135 140

Glu Pro Pro Ala Asn Val Val Phe Ile Leu Ala Thr Thr Glu Pro His
145 150 155 160

Lys Ile Pro Ala Thr Ile Ile Ser Arg Thr Gln Arg Phe Asp Phe Lys
165 170 175

Arg Ile Asp Asn Gln Asp Ile Ile Asp Arg Leu Ile Tyr Ile Leu Glu
180 185 190

Glu Asp Gln Val Pro Tyr Ser Lys Glu Ala Val Leu Ser Leu Ala Asn
195 200 205

Ala Ala Glu Gly Gly Met Arg Asp Ala Leu Ser Met Leu Asp Gln Ala
210 215 220

Leu Ser Phe Met Thr Asp Glu Leu Thr Glu Glu Val Ala Leu Gln Ile
225 230 235 240

Thr Gly Ser Ile Thr Gln Ser Leu Leu Leu Glu Tyr Leu Gln Val Ile
245 250 255

Ser Gln Gly Gln Thr Glu Glu Gly Leu Lys Leu Leu Gln Glu Val Leu
260 265 270

Gly Glu Gly Lys Asp Pro Ser Arg Phe Val Glu Asp Ala Ile Met Met
275 280 285

Thr Arg Asp Leu Leu Leu Tyr Gln Thr Ser Gln Gly Asp Asn Phe Val
290 295 300

Pro Lys Leu Ala Arg Leu Asp Asp Gln Phe Glu Asp Leu Ala Lys Asp
305 310 315 320

Leu Asp Lys Glu Met Ala Tyr His Ile Ile Asp Val Leu Asn Gln Thr
325 330 335

Gln Asp Asp Leu Arg Leu Ser Asn His Gly Glu Val Tyr Leu Glu Ile
340 345 350

Ala Thr Val Lys Leu Ser Gln Pro Ser Ser Ala Val Gln Thr Ile Gln
355 360 365

Ala Ser Gln Val Asn Met Val Asp Gln Asp Asn Lys Glu Glu Ile Ala
370 375 380

Gln Leu Gln Asn Gln Val Lys Ser Leu Gln Gln Ser Ile Gln Asn Leu
385 390 395 400

Gln Ala Gly Ala Lys Gln Gly Pro Lys Gln Arg Ala Lys Ser Lys Ala
405 410 415

Gly Pro Lys Gln Ser Gly Pro Gly Lys Ser Arg Ser His Arg His Gln
420 425 430

Gln Gly Phe Lys Val Asn Arg Lys Ala Val Tyr Ser Ile Leu Asp Gln
435 440 445

Ala Thr Arg Lys Asp Leu Asp Asp Leu Gln Asp Leu Trp Pro Asp Leu
450 455 460

Ile Asn Val Leu Thr Ile Ser Gln Lys Ala Ile Leu Asn Asn Ser Lys
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Pro Val Ala Ala Ser Pro Glu Gly Leu Val Val Thr Phe Glu Tyr Asp
485 490 495

Ile Leu Cys Glu Arg Ala Glu Ser Asp Glu Thr Leu Gln Thr Ala Ile
500 505 510

Gly Asn Tyr Ile Glu Lys Ile Ile Gly Arg Arg Pro Arg Leu Val Cys
515 520 525

Val Pro Glu Asp Lys Trp Pro Thr Ile Arg Arg Asp Phe Ile Lys Gln
530 535 540

Met Lys Lys Glu Asp Gly Ser Thr Lys Ala Gly Gln Ala Ser Asp Gly
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Lys Ser Asp Asp Asp Pro Gly Gln Glu Asp Asn Gln Ala Leu Asn Lys
565 570 575

Ala Val Glu Leu Phe Gly Lys Asp Asn Ile Thr Ile Lys Asp
580 585 590